PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/28, 38/54, 39/395, 45/05

A1

(11) International Publication Number: **WO 99/43343**

(43) International Publication Date: 2 September 1999 (02.09.99)

(21) International Application Number: PCT/US99/04218

(22) International Filing Date: 26 February 1999 (26.02.99)

(30) Priority Data:
09/031,279 27 February 1998 (27.02.98) US

(71) Applicant *(for all designated States except US)*: SUPRATEK PHARMA INC. [CA/CA]; Suite 700, 275 St. Jacques, Montreal, Quebec H2Y 1M9 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KABANOV, Alexander, V. [RU/US]; 1304 South 126th Street, Omaha, NE 68144 (US). ALKHOV, Valery, Y. [-/CA]; 22 Sunny Acres Baie, D'Urfe, Quebec H9X 3B6 (CA).

(74) Agent: BERNSTEIN, Scott, N.; Mathews, Collins, Shepherd & Gould, P.A., Suite 306, 100 Thanet Circle, Princeton, NJ 08540 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NOVEL PEPTIDE COPOLYMER COMPOSITIONS

(57) Abstract

Compositions of peptides and block copolymers and methods of treatment using the same. The compositions enhance the activity of peptide-based and related biological agents, and reduce adverse side effects.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

NOVEL PEPTIDE COPOLYMER COMPOSITIONS

This application is a continuation-in-part of U.S. Application No. 08/478,979, filed June 7, 1995, and a continuation-in-part of U.S. Application No. 08/951,079, filed October 15, 1997, which is a divisional of U.S. Application No. 08/478,978 filed June 7, 1995, which is a continuation-in-part of 08/374,406, filed January 17, 1995, which in turn is a continuation of U.S. Application No. 07/957.998, filed October 8, 1992.

FIELD OF THE INVENTION

The invention relates to copolymer pharmaceutical compositions useful in administering a number of peptide-based biological agents.

BACKGROUND OF THE INVENTION

A variety of peptide-based and related biological agents are currently in use for the treatment of diseases and disorders. While many biological agents have proven somewhat useful in the treatment of such diseases and disorders, many therapies are accompanied by adverse side effects, difficulty in administering the agent or agents to the desired target site, multi-drug resistance (MDR), as well as difficulty in crossing the blood-brain barrier.

Peptides are molecules consisting of two or more amino acids. Shorter peptides (of three or more amino acids) are termed oligopeptides. Longer peptide chains are termed polypeptides. Proteins are macromolecule polypeptides, and this includes such molecules as enzymes, hormones, antibodies, and the like.

Blood-Brain Barrier

The brain is isolated from circulatory blood because the endothelial cell lining of blood vessels in the brain is more selective than it is in other parts of the body with respect to the molecules that are allowed to diffuse into the interstitial space of the brain. The mechanism that isolates the brain is often referred to as a

"blood-brain barrier." As a result of the blood-brain barrier, biological agents that are intended to affect the brain or a disease in the brain often must be administered in high dosage to compensate for the diffusion barrier provided by the blood-brain barrier. Animals to whom the high doses are administered are at greater risk of experiencing toxic or other side effects. It is therefore desirable to enhance the permeability of chemotherapeutic agents across the blood-brain barrier. See, *Goodman's and Gilman's The Pharmacological Basis of Therapeutics,* 8th Ed., p.11.

In the brain and in other tissues it is often desirable to target a biological agent to a particular tissue at which the agent is anticipated to beneficially act. This desirability is particularly true for chemotherapeutic agents that potentially have highly toxic effects on non-target tissues. For instance, most anti-cancer chemotherapeutic agents function by selectively poisoning replicating cells. This mechanism inevitably targets the rapidly replicating cells, such as those of the bone marrow that generate a number of important blood cells. If the biodistribution of the chemotherapeutic drug is changed so that useful concentrations are maintained in the cancerous tissue or the tissue in which the cancer resides while concentrations distal from the cancer *situs* are reduced, the scope of toxic side effects will generally be reduced.

Additionally, since cancer, antimicrobial and other biological agents exhibit toxicities, it would be beneficial if dosages were lowered without adversely affecting the therapeutic index.

Cancer

Tumors of the central nervous system present a particularly difficult therapeutic challenge. Such tumors are often difficult to surgically excise and surgical excision can have unacceptable consequences. These tumors can be difficult to treat with radiation since they are sometimes difficult to precisely locate and are often too close to tissues that are critical to the well-being of the tumor patient. Such tumors cannot be effectively treated by standard chemotherapies since the fraction of the administered chemotherapeutic agent that

will reach the tumor is very small. The effective dosage at the tumor cannot be increased by administering higher dosages to the patient, since standard dosages are generally close to the dose that cause unacceptable side effects.

Cytokines

Cytokines are polypeptides secreted by cells. Cytokines play an important role in the interactions between cells in the immune system, and are therefore potentially effective drugs for the treatment of cancer, as well as viral-related and other diseases. The mechanism of action of these protein factors is connected with specific activation of the immune system which, in turn, protects against many pathological processes. Well known are antiviral preparations on the-basis of interferons (Infs) that are already used in clinical practice. For example, clinical tests of interleukin-2 (IL-2) and tumor necrosis factor (TNF) as anticancer drugs have yielded promising results. A great deal of work has been devoted to creation of new drugs on the basis of IL-4 and other lymphokines.

Generally speaking, recombinant cytokines possess low affinity for specific receptors on target cells because of incorrectly formed tertiary structures and the absence of necessary post- translational modifications in bacterial super-producers. Such recombinant preparations display low biological activity, and very high doses are required, producing considerable side effects.

Hormones

Hormones are chemical messenger molecules secreted by endocrine glands which regulate various aspects of metabolism. Insulin, for example, is a protein hormone secreted in the pancreas by the islets of Langerhans. Insulin stimulates catabolism of glucose and blocks glycogenolysis, thereby facilitating diffusion of glucose into most cells. The inability to form insulin results in diabetes mellitus, which is currently treated through insulin injection in conjunction with dietary regulation to control blood sugar levels. Insulin production and thus is of particular interest in molecular biology and enzymology.

SUMMARY OF THE INVENTION

It has now been found that the activity of peptide-based and related biological agents can be enhanced, and adverse side effects reduced, by the administration of such peptides in conjunction with a block copolymer.

In one embodiment, the invention provides a pharmaceutical composition comprising:

(a) a biological agent;

(b) a polyether block copolymer comprising an A-type linear polymeric segment joined at one end to a B-type linear polymeric segment, wherein the A-type segment is of relatively hydrophilic character, the repeating units of which have molecular weight contributions between about 30 and about 500, wherein the B-type segment is of relatively hydrophobic character, the repeating units of which have molecular weight contributions between about 30 and about 500, wherein at least about 80% of the linkages joining the repeating units for each of the polymeric segments comprise an ether linkage; and

(c) a targeting moiety coupled to a lipophilic moiety comprising a hydrocarbon having from about 3 to about 41 carbon atoms, more preferably a hydrocarbon having from about 5 to about 25 carbon atoms, and more preferably, a hydrocarbon having from about 9 to about 17 carbon atoms.

The invention thus relates to pharmaceutical compositions comprising a biological agent and a poly(oxyethylene)-poly(oxypropylene) block copolymer. Preferred compositions include those wherein the poly(oxypropylene) [*i.e.*, hydrophobe] portion of said block copolymer comprises at least 50% by weight of the block copolymer. Also preferred are compositions wherein the hydrophobe molecular weight of the block copolymer is at least about 900, and more preferably at least about 1700. Especially preferred are compositions wherein the hydrophobe molecular weight of the polyether block copolymer is at least about

2000 and the hydrophobe weight percentage is at least about 20%. The invention also relates to methods of treatment using the same.

Also preferred are compositions wherein the block copolymers have a critical micellar concentration ("CMC") of about 0.5% wt/vol or less at 37°C in an isotonic aqueous solution.

Additionally preferred are compositions wherein the biological agent is a peptide, or derivative thereof. This includes oligopeptides, polypeptide, proteins, enzymes, hormones, or cytokines.

In yet another preferred embodiment, the polyether block copolymer is selected from the group consisting of polymers of formulas:

A-B-A', A-B, B-A-B', or $L(R')(R^2)(R^3)(R^4)$

(I) (II) (III) (IV)

wherein A and A' are A-type linear polymeric segments, B and B' are B-type linear polymeric segments, and R^1, R^2, R^3 and R^4 are either block copolymers of formulas (I), (II) or (III) or hydrogen and L is a linking group, with the proviso that no more than two of R^1, R^2, R^3 or R^4 are hydrogen.

In a preferred embodiment, the composition is adapted to include micelles composed of the block copolymer or to form micelles composed of the block copolymers during the course of administration or subsequent thereto. Preferably, at least about 0.1% of the biological agent is incorporated in the micelles, more preferably, at least about 1% of the biological agent, yet more preferably, at least about 5% of the biological agent.

In a preferred embodiment, the hydrophobe percentage of the copolymer of the composition is at least about 50% more preferably, at least about 60%, yet more preferably 70%.

In another preferred embodiment, the hydrophobe weight of the copolymer is at least about 900, more preferably, at least about 1700, yet more preferably at least about 2000, still more preferably at least about 2300.

In further preferred embodiments, the hydrophobe weight is at least about 2000 and the hydrophobe percentage is at least about 20%, preferably 35%; or the hydrophobe weight is at least about 2300 and the hydrophobe percentage is at least about 20%, preferably 35%.

In yet another preferred embodiment, the copolymer or copolymers of the composition have a critical micellar concentration ("CMC") of no more than about 0.5% wt/vol at 37°C in an isotonic aqueous solution, preferably, no more than about 0.05% wt/vol., more preferably, no more than about 0.01% wt/vol., yet more preferably, no more than about 0.003% wt/vol.

Preferably, the copolymers of the composition conform to Formula (V), which is set forth in the text below. Particularly preferred among these copolymers are those having hydrophobe weights between about 1500 and about 2000, preferably between about 1710 and about 1780, and hydrophobe percentages between about 85% and about 95%, preferably between about 88% and about 92%. Also particularly preferred among these copolymers are those having hydrophobe weights between about 3000 and about 3500, preferably between about 3200 and about 3300, and hydrophobe percentages between about 15% and about 25%, preferably between about 18% and about 22%. Additionally particularly preferred among these polymers are that having hydrophobe weights between about 3500 and about 4000, preferably between about 3700 and about 3800, and hydrophobe percentages between about 25% and about 35%, preferably between about 28% and about 32%.

In a preferred embodiment, the biological agent of the composition is an agent that affects the function of the brain or treats or prevents a disease of the brain.

In a second embodiment, the invention provides a pharmaceutical composition comprising a biological agent solubilized in polymeric micelles

having associated therewith a targeting moiety coupled to a lipophilic moiety comprising hydrocarbon having from about 3 to about 41 carbon atoms, more preferably a hydrocarbon having from about 5 to about 25 carbon atoms, yet more preferably, a hydrocarbon having from about 9 to about 17 carbon atoms.

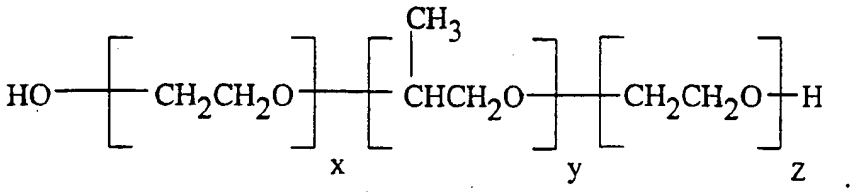
In another embodiment, the invention provides a method of targeting a biological agent to a pre-selected tissue. The method comprises administering the composition described above, wherein the targeting moiety is selected to target the tissue, to an animal having the pre-selected tissue.

In yet another embodiment, the invention provides a method of treating a microbial disease or a tumor of the brain by administering a composition comprising:

(a) a chemotherapeutic agent; and

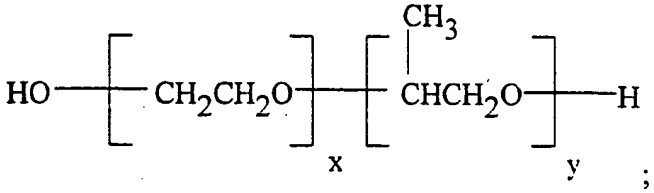
(b) a polyether block copolymer comprising an A-type linear polymeric segment joined at one end to a B-type linear polymeric segment, wherein the A-type segment is of relatively hydrophilic character, the repeating units of which contribute an average Hansch-Leo fragmental constant of about 0.4 or less and have molecular weight contributions between about 30 and about 500, wherein the B-type segment is of relatively hydrophobic character, the repeating units of which contribute an average Hansch-Leo fragmental constant of about -0.4 or more and have molecular weight contributions between about 30 and about 500, wherein at least about 80% of the linkages joining the repeating units for each of the polymeric segments comprise an ether linkage. In a preferred embodiment, the composition used in this embodiment will include a targeting molecule.

In yet another embodiment, the present invention relates to a composition comprising a poly(oxyethylene)-poly(oxypropylene) block copolymer and a protein, peptide, or derivative thereof covalently modified with a hydrophobe. The preferred block copolymers are of the formula:



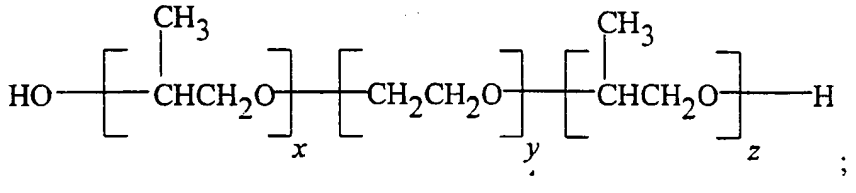
;

(V)



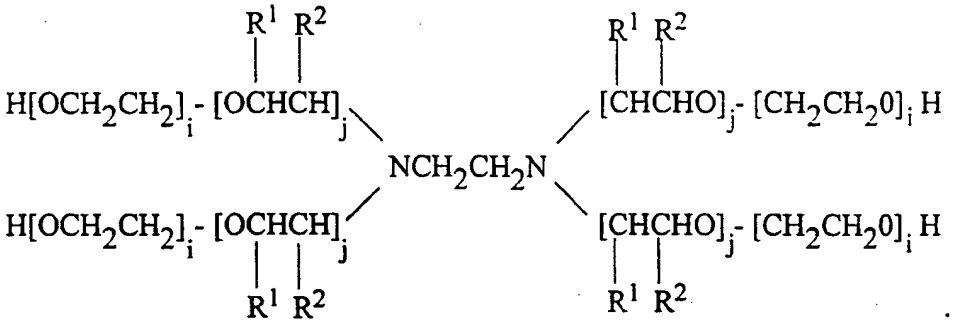
;

(VI)



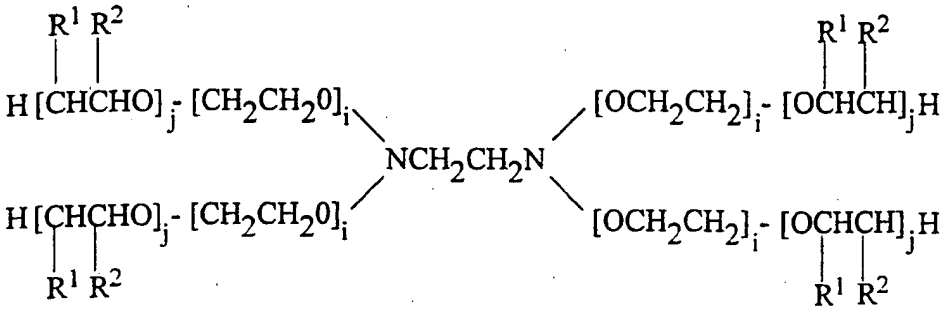
;

(VII)



; or

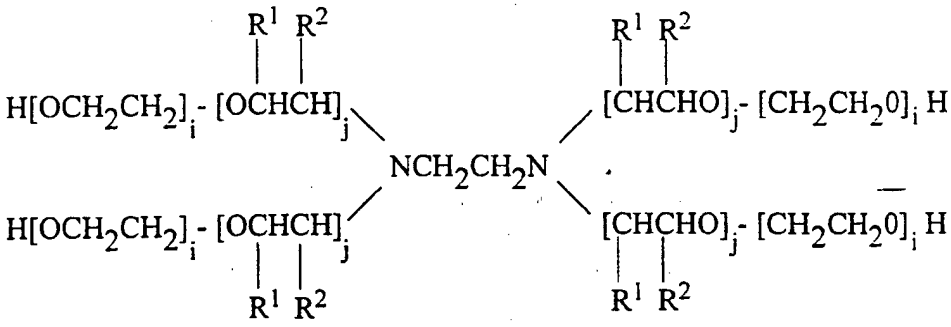
(VIII)



(VIV)

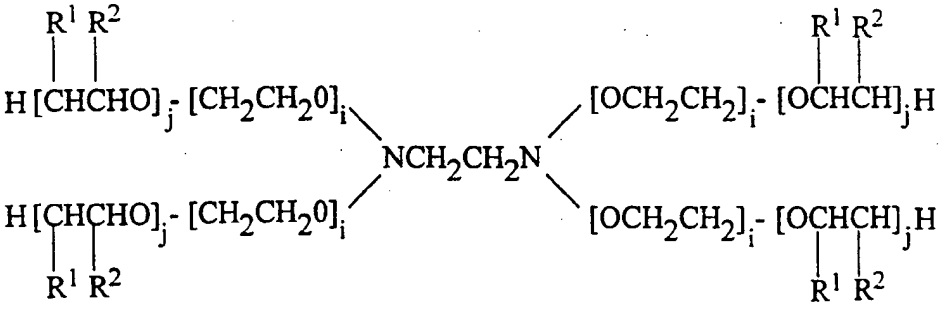
in which *x*, *y*, *z*, *i*, and *j* have values from about 2 to about 400, and wherein for each R^1, R^2 pair, one is hydrogen and the other is a methyl group.

In another preferred embodiment, the block copolymer is of the formula:



; or

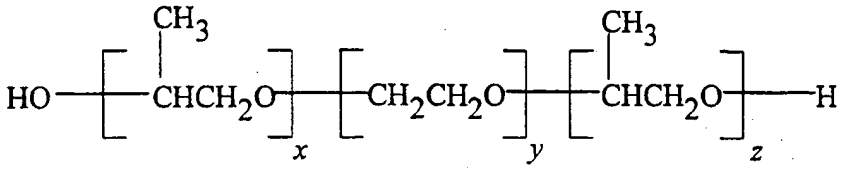
(VIII)



(VIV)

wherein for each R^1, R^2 pair, one is hydrogen and the other is a methyl group. Preferred are those block copolymers wherein the ethylene(oxide) content of said block copolymer is less than 50%.

The invention also relates to a composition comprising a protein, peptide, or derivative thereof, and a POE-POP block copolymer of the formula:



(VII)

in which x, y, and z have values from about 2 to about 400.

In yet another preferred embodiment, the invention relates to compositions comprising at least one block copolymer with an ethylene(oxide) content of 50% or less, and at least one block copolymer with ethylene(oxide) content of 50% or more.

Preferred hydrophobes include lipids, and fatty acid residues.

Preferred protein, peptide, or derivatives are those with a molecular weight of at least about 1,000 Daltons, more preferably at least about 5,000 Daltons, even more preferably at least about 10,000 Daltons.

Preferred proteins, peptides, or derivatives thereof include immunomodulators, cytokines, hormones, enzymes, tissue plasminogen activators, clotting factors, colony stimulating factors, neuropeptides (or derivative thereof), recombinant soluble receptors, monoclonal antibodies, and erythropoetins.

Preferred hormones include human growth hormone.

The invention also relates to methods of treatment comprising administering to a patient the above poly(oxyethylene)-poly(oxypropylene) block copolymers and a protein, peptide or derivative thereof, covalently modified with a hydrophobe.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The terms or phrases listed below shall have the following meaning:

- Biological agent: An agent that is useful for diagnosing or imaging or that can act on a cell, organ or organism, including but not limited to drugs (pharmaceuticals) to create a change in the functioning of the cell, organ or organism. Such agents can include but are not limited to peptides and polypeptides, nucleic acids, polynucleotides, antibacterial agents, antiviral agents, antifungal agents, anti-parasitic agents, tumoricidal or anti-cancer agents, proteins, toxins, enzymes, hormones, neurotransmitters, glycoproteins, immunoglobulins, immunomodulators, dyes, radiolabels, radio-opaque compounds, fluorescent compounds, polysaccharides, cell receptor binding molecules, anti-inflammatories, anti-glaucomic agents, mydriatic compounds and local anesthetics, and biological agents that act on cells of the central nervous system or diseases of the central nervous system.

- Central nervous system agents: Biological agents that act on cells of the central nervous system or diseases of the central nervous system.

- Chemotherapeutic agent: A biological agent that inhibits the growth or decreases the survival of neoplastic or pathogenic microbial cells or inhibits the propagation (which includes without limitation replication, viral assembly or cellular infection) of a virus.

- Hydrophobe percentage: The percentage of the molecular weight of a block copolymer that is made up of B-type blocks.

- Hydrophobe weight: The molecular weight contribution of the B-type blocks of a block copolymer.

· IC_{50}: The concentration at which 50% cytotoxicity is obtained. Cytotoxicity can be measured by the method of Alley *et al.*, *Cancer Res.*, 48: 589-601 (1988) or Scudiero *et al.*, *Cancer Res.*, 48:4827 (1988). In particular, it can be measured based on the drug concentration at which a 50% reduction in the activity of mitochondrial enzymes is observed.

IC_{95}: The concentration at which 95% cytotoxicity is obtained. Cytotoxicity can be measured by the method of Alley *et al.*, above, or Scudiero *et al.*, above. Specifically, it can be measured based upon the drug concentration at which a 95% reduction in the activity of mitochondrial enzymes is observed.

· Lipophilic moiety: A lipophilic substituent that is joined to a targeting moiety and that partitions into the lipophilic portion of copolymer micelles.

· MDR: The phenomenon of simultaneous resistance to unrelated biological agents.

· Microbe: A bacteria, mycoplasma, yeast or fungi, virus or parasite (such as a malaria parasite).

· Targeting moiety: A molecular structure that is recognized by a cellular, tissue, viral or substratum component such as a cell surface receptor or acceptor molecule.

It will be understood that the copolymer characteristics described below are suitable for the compositions of both the targeting embodiments of the invention and the brain chemotherapy embodiments of the invention.

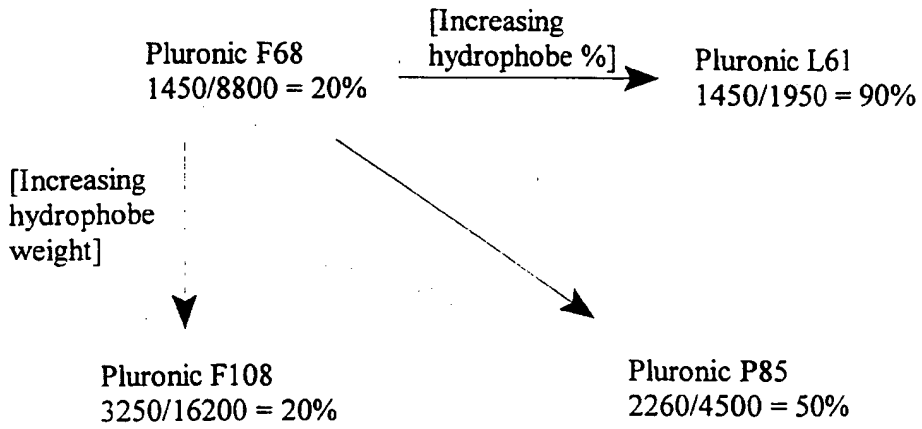
The mechanism by which the blood-brain barrier works is believed to be substantially similar to the mechanism by which many cells become resistant to the action of biological agents. Both mechanisms are believed to make use of the membrane pump proteins belonging to the glycoprotein-P family of proteins. See,

for example, Tatsuta *et al.*, *J. Biol. Chem.*, 267:20383-20391, and Goldstein *et al.*, *Cancer Treatment Res.*, 57:101-119. These pumps are believed to act by exporting biological agents that diffuse into a cell, such as the endothelial cells that line blood vessels in the brain. Recent observations described in more detail in U.S. Application No. 08/478,978 filed June 7, 1995, entitled "Biological Agent Compositions", demonstrate the effectiveness of the block copolymers of the invention in enhancing the potency of chemotherapeutic drugs and reversing drug resistance is highly dependent (a) on the hydrophobe percentage and (b) on the hydrophobe weight. The effectiveness increases with either an increase in the percentage (a) or an increase in weight (b), or both. These hydrophobe percentage and hydrophobe weight increases also correlate with improved micelle formation properties wherein micelle formation for these copolymers occurs at lower concentrations. See, Hunter *et al.*, *Macromolecules* 26: 5030 (1993); Hunter *et. al.*, *Macromolecules* 26: 5592 (1993); Alexandris *et. al.*, *Macromolecules* 27: 2414 (1994).

While not wishing to be limited to a particular theory, it is believed that micelle formation serves as a surrogate for measuring the physical properties that lead to improved biological agent delivery properties.

If, using doxorubicin as a model biological agent, the ratio of (a) the IC_{50} (a measure of effective cytotoxicity concentration) for a copolymer-containing composition to (b) the IC_{50} for free doxorubicin is plotted against the concentration of copolymer, the plot is biphasic, with a rapid decrease in the ratio seen as copolymer concentrations increase but remain under the CMC of the copolymer. Above the CMC, a rapid leveling off of the ratio is observed. Maximal enhancement of biological agent activity occurs above the CMC, although enhancement activity is seen at concentrations, for the copolymer Pluronic L61, as low as 0.0001 % wt/vol., or less. The micellar form is also believed to be important to using the copolymers in drug delivery for other reasons, as will be discussed below.

The schematic below is helpful in understanding the relationship between the hydrophobe percentage and the hydrophobe weight of a copolymer and various aspects of the present invention. In the schematic, the weight of the hydrophobe (poly(oxypropylene)) and of the copolymer are shown directly under each identified copolymer. Adjacent to these values are the hydrophobe percentage values for each copolymer.



Pluronic F68 has been determined to have only a modest activity in enhancing the potency of biological agents. Pluronic L61, which has the same hydrophobe weight as Pluronic F68 but a much higher hydrophobe percentage, is generally the most effective of the block copolymers identified in the schematic. Pluronic F108, which has the same hydrophobe percentage as Pluronic F68 but a much higher hydrophobe weight, is also an effective copolymer, though much less effective than Pluronic L61. Pluronic P85 has a greater hydrophobe weight and a greater hydrophobe percentage than Pluronic F68, but the difference in each value is less than it is for Pluronics F108 and L61, respectively. The effectiveness of Pluronic P85 in enhancing the potency of biological agents is intermediate between the effectiveness of Pluronic F108 and of Pluronic L61. These differences in effectiveness are exemplified when various copolymers, at a concentration above CMC, and doxorubicin are incubated *in vitro* with drug resistant cells. The ratio of the IC50 value for doxorubicin in the absence of copolymer to the ratio in the

presence of copolymer is the "resistance reversion index." The resistance reversion index values for various copolymers are:

Doxorubicin formulation	IC_{50}, ng/ml	Resistance reversion index
free drug	60,000	n.a.
+ 5% (w/v) Pluronic F68	60,000	1
0.01% (w/v) Pluronic F108	10,000	6
0.01% (w/v) Pluronic P85	2,000	30
0.01% (w/v) Pluronic L61	60	1000

The importance of the micellar form in delivering biological agents is also revealed in *in vivo* experiments. In micellar form, biological agents are located in the hydrophobic core of the micelles, thereby masked by the hydrophilic shell (composed of A-type segments) surrounding the micelles. This masking decreases interactions with liver, plasma proteins, other non-target tissues and other molecules that can bind or inactivate the agent or convert the agent to a toxic metabolite. For example, rapid metabolism of anthracycline antibiotics by the liver leads to the formation of cardiotoxic metabolites that are modified at the C13 position. *See*, Mushlin, *et al.*, *Br. J. Pharmacol.*, 110: 975-982 (1993). Using doxorubicin as a model drug, the micellar form decreases liver uptake, decreases conversion to doxorubicinol, and decreases the rate at which the concentration of doxorubicin in the blood decreases.

The effectiveness of copolymers in (a) forming micelles (where greater effectiveness is measured in reduced CMCS) and (b) favoring the partitioning of various biological agents to the micellar rather than the free form of various biological agents increases according to the same pattern. Thus, the hierarchy of effectiveness is again L61 > P85 > F108 >> F68. The presence of micelles at low concentrations is believed to help assure, assuming that biological agent remains associated with the micelles, that the biological agent and the copolymer arrive together at a target tissue. Partitioning coefficients that favor the micellar form help assure that the assumption that the biological agent remains associated with

micelles will hold true. The micellar form of the biological agent is also believed to protect the biological agent from uptake by non-target tissues, which tissues may metabolize the biological agent into an ineffective or toxic metabolite, and non-specific adsorption to blood components, cellular components and the like.

At high concentrations, block copolymers can be toxic to the liver, kidney or other cells of a subject. *See, BASF Corp., Pluronic Material Safety Data Sheet and Drug Master Files.* The toxicity of block copolymers increases with the hydrophobicity parameters of block copolymers according to the same pattern seen for increases in effectiveness in potentiating biological agents. Fortunately, the rate of increase in potency as these hydrophobicity parameters change is much greater than the increase in copolymer toxicity. For instance, the LD_{50} of L61 in BALB/c mice is 10-fold lower than the LD_{50} of Pluronic F108. However, the difference in the optimal therapeutic dose is more than 100-fold improved for Pluronic L61 vs. Pluronic F108 (see Example 14). Thus, the concentration range over which effectiveness in potentiating the activity of a biological agent can be maintained while avoiding toxicity due to copolymer is increased for Pluronic L61 vs. Pluronic F108.

Without wishing to be bound to a particular theory, it is believed that the compositions of the invention reverse efflux mechanisms mediated by members of the glycoprotein-P family and other drug resistance mechanisms.

The compositions of the invention are intended to include either preformed micelles with a substantial portion of the biological agent incorporated therein, or copolymer compositions which form micelles with a substantial portion of the agent dissolved therein during the course of the administration of the biological agent to a patient, or subsequent thereto. For the targeting embodiment of the invention, the targeting moiety will either be pre-associated with micelles or will associate with micelles during the course of administration. Particularly preferred block copolymers are those that have low CMC values in isotonic solutions at physiological temperatures. Such block copolymers will maintain a micellar delivery vehicle for biological agents even after substantial dilution into a

physiological fluid such as a treatment subject's blood. Such low CMC values allow for the use of reduced levels of block copolymers in the drug composition of the invention.

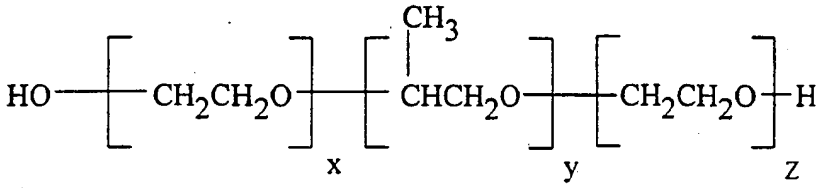
The invention is described below with reference to the fragmental constants developed by Hansch and Leo. See Hansch and Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley,* New York, 1979; James, *Solubility and Related Properties,* Marcel Dekker, New York, 1986, pp. 320-325. These constants were developed for use in estimating the contribution of a portion of a molecule to the tendency of the molecule to partition between the phases formed by octanol-water mixtures. These constants are generally referred to as Hansch-Leo fragmental partition constants (hereinafter "Hansch-Leo fragmental constants").

The entire disclosure of U.S. Application No. 08/478,978 filed June 7, 1995, entitled, "Biological Agent Compositions", filed concurrently herewith on June 7, 1995 is incorporated herein by reference, as is the entire disclosure of U.S. Patent Application No. 08/054,403, filed April 28, 1993.

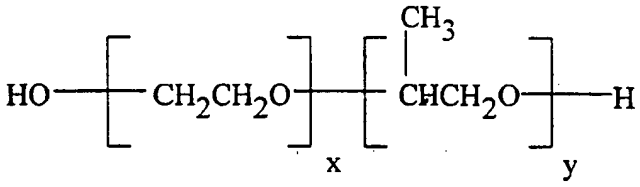
The number of repeating units of the total hydrophilic (A-type) blocks or the total hydrophobic (B-type) blocks for a polyether copolymer are I preferably be between about 4 and about 400. More preferably, the number of repeating units is between about 4 and about 200, still more preferably, between about 5 and about 80. The repeating units that comprise the blocks, for A-type and B-type blocks, will generally have molecular weight between about 30 and about 500, preferably between about 30 and about 100, still more preferably between about 30 and about 60. Generally, in each of the A-type or B-type blocks, at least about 80% of the linkages between repeating units will be ether linkages, preferably, at least about 90% will be ether linkages, more preferably, at least about 95% will be ether linkages. Ether linkages, for the purposes of this application, encompass glycosidic linkages (*i.e.*, sugar linkages). However, in one aspect, simple ether linkages are preferred.

Preferably, all of the repeating units that comprise A-type blocks have a Hansch-Leo fragmental constant of less than about -0.4, more preferably, less than about -0.5, still more preferably, less than about -0.7. Preferably, all of the repeating units that comprise B-type blocks have a Hansch-Leo fragmental constant of about -0.30 or more, more preferably about -0.20 or more.

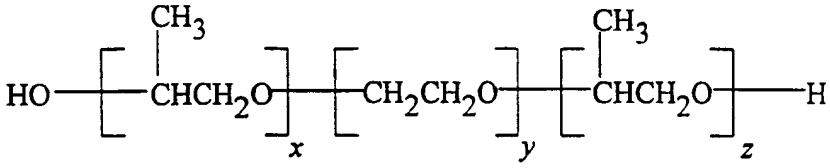
Polymers according to the first embodiment of the invention are exemplified by the block copolymers having the formulas:



(V)

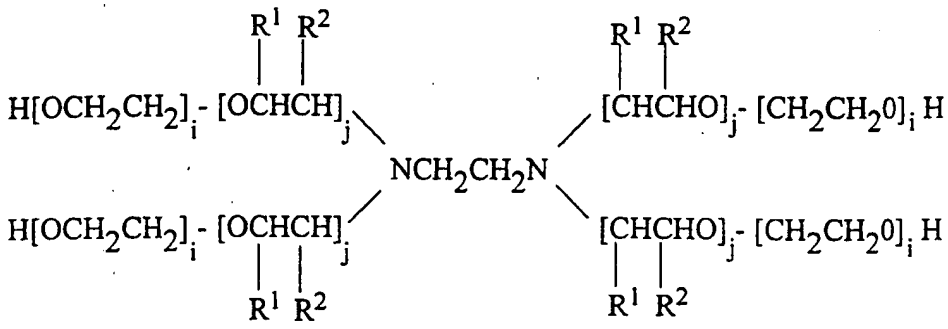


(VI)

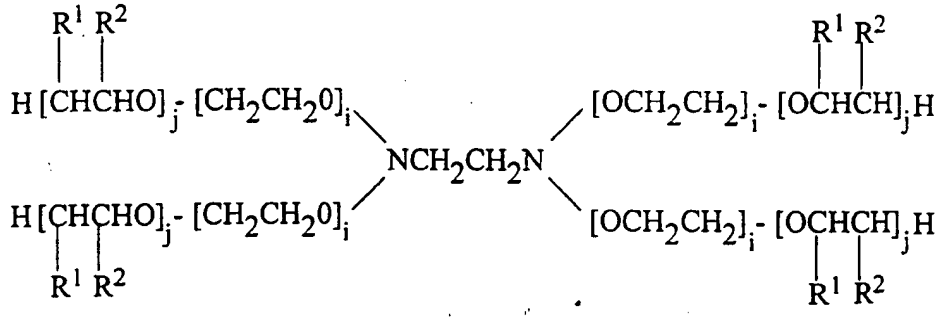


(VII)

or,



(VIII)



(VIV)

in which *x*, *y*, *z*, *i*, and *j* have values from about 2 to about 400, preferably from about 5 to about 200, more preferably from about 5 to about 80, and wherein for each R^1, R^2 pair, one is hydrogen and the other is a methyl group. Formulas (V) through (VII) are oversimplified in that, in practice, the orientation of the isopropylene radicals within the B block will be random. This random orientation is indicated in formula (VIII), which is more complete. Such poly(oxyethylene)-poly(oxypropylene) compounds have been described by Santon, *Am. Perfumer Cosmet.*, 72(4):54-58 (1958); Schmolka, *Loc. cit.* 82(7):25-30 (1967); *Non-ionic Surfactants,* Schick, ed. (Dekker, N.Y., 1967), pp. 300-371. A number of such compounds are commercially available under such generic trade names as "lipoloxamers", "pluronics" and "synperonics." Pluronic polymers within the B-A-B formula are often referred to as "reversed" pluronics, "pluronic R" or "meroxapol."

The "polyoxamine" polymer of formula (VIII) is available from BASF (Wyandotte, MI) under the tradename Tetronic™. The order of the polyoxyethylene and polyoxypropylene blocks represented in formula (VIII) can be reversed, creating Tetronic-R™, also available from BASF. See, Schmolka, *J. Am. Oil. Soc.*, 59:110 (1979). Polyoxypropylene-polyoxyethylene block copolymers can also be designed with hydrophilic blocks comprising a random mix of ethylene oxide and propylene oxide repeating units. To maintain the hydrophilic character of the block, ethylene oxide will predominate. Similarly, the hydrophobic block can be a mixture of ethylene oxide and propylene oxide repeating units. Such block copolymers are available from BASF under the tradename Pluradot™.

The hydrophobic/hydrophilic properties of a given block copolymer depends upon the ratio of the number of oxypropylene groups to the number of oxypropylene groups. For a composition containing a single block copolymer of poly(oxyethylene)-poly(oxypropylene), for example, this relationship, taking into account the molecular masses of the central hydrophobic block and the terminal hydrophilic blocks, can be expressed as follows:

$$n = \frac{H}{L} \bullet 1.32$$

in which H is the number of oxypropylene units and L is the number of oxyethylene units. In the general case of a block copolymer containing hydrophobic B-type segments and hydrophilic A-type segments, the hydrophobic-hydrophilic properties and micelle-forming properties are related to the value n as defined as:

$$n = (|B|/|A|) \times (b/a)$$

where |B| and |A| are the number of repeating units in the hydrophobic and hydrophilic blocks of the copolymer, respectively, and b and a are the molecular weights for the respective repeating units.

Selecting a block copolymer with the appropriate n value depends upon the hydrophobic/hydrophilic properties of the specific agent, or the composite hydrophilic/hydrophilic properties of a mixture of agents to be formulated. Typically, n will range in value from about 0.2 to about 9.0, more preferably between about 0.25 and about 1.5. This range should be viewed not as numerically critical but as expressing the optimum hydrophobic/hydrophilic balance between the predominantly hydrophilic poly(oxyethylene) blocks, and the predominantly hydrophobic poly(oxypropylene) blocks.

An important aspect of the present invention-involves utilizing mixture of different block-copolymers of poly(oxyethylene)-poly(oxypropylene) to achieve a more specific hydrophobic-hydrophilic balance suitable for a given cytokine or mixture of several cytokines, preserving the optimal size of particles. For example, a first block copolymer may have an n of 1.0 whereas a second may have a value of 1.5. If material having an n of 1.3 is desired, a mixture of one weight portion of the first block copolymer and 1.5 weight portion of the second block-copolymer can be employed.

Thus, a more generalized relationship for such mixtures can be expressed as follows:

$$N = 1.32 \bullet \left[\frac{H_1 \bullet m_1}{(L_1) \bullet (m_1 + m_2)} + \frac{H_2 \bullet m_2}{(L_2) \bullet (m_1 + m_2)} \right]$$

in which H_1 and H_2 are the number of oxypropylene units in the first and second block copolymers, respectively; L_1 is the number of oxyethylene units in the first block copolymer; L_2 is the number of oxyethylene units in the second block copolymer; m_1 is the weight proportion in the first block-copolymer; and m_2 is the weight proportion in the second block copolymer.

An even more general case of a mixture of K block copolymers containing hydrophobic B-type block copolymers and hydrophilic A-type block copolymers, the N value can be expressed as follows:

$$N=\frac{b}{a}\sum_{i=1}^{k}\left(\frac{|B|_i}{|A|_i},\frac{m_i}{M}\right)$$

where $|A|_i$ and $|B|_i$ are the numbers of repeating units in the hydrophilic (A-type) and hydrophobic (B-type) blocks of the i-th block copolymer, m is the weight proportion of this block copolymers, M is the sum of weight proportions of all block copolymers in the mixture ($M = \sum_{i=1}^{k} m_i$), and a and b are the molecular weights for the repeating units of the hydrophilic and hydrophobic blocks of these block copolymers respectively.

If only one block copolymer of poly(oxyethylene)-poly(oxypropylene) is utilized, N will equal n. An analogous relationship will apply to compositions employing more than two block copolymers of poly(oxyethylene)-poly(oxypropylene).

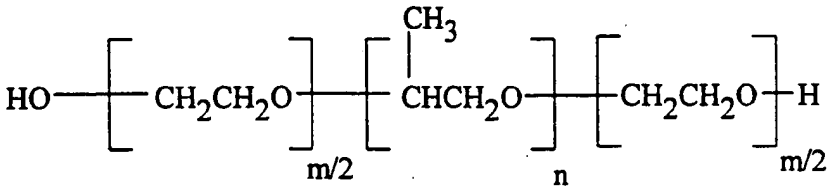
Where mixtures of block copolymers are used, a value N will be used, which value will be the weighted average of n for each contributing copolymers, with the averaging based on the weight portions of the component copolymers. The value N can be used to estimate the micelle-forming properties of a mixture of copolymers. The use of the mixtures of block copolymers enhances solubility and prevents aggregation of more hydrophobic block copolymers in the presence of the serum proteins. Particularly, poly(oxyethylene)-poly(oxypropylene) block copolymers with the ethylene oxide content of more than 50% solubilize hydrophobic block copolymers with ethylene oxide content of no more than 50%. In such mixtures, the preferred ratio of the hydrophilic and hydrophobic copolymer is at least 2:1 (w/w), preferably at least 5:1 (w/w), still more preferably at least 8:1 (w/w)." When copolymers other than polyethylene oxide-polypropylene oxide copolymers are used, similar approaches can be developed to

relate the hydrophobic/hydrophilic properties of one member of the class of polymers to the properties of another member of the class.

Using the above parameters, one or more block copolymers of poly(oxyethylene)-poly(oxypropylene) are combined so as to have a value for *N* of from about 0.1 to about 9, more preferably from about 0.25 to about 1.5. The combined copolymers form micelles, the value of *N* affecting in part the size of the micelles thus produced. Typically the micelles will have an average diameter of from about 10 to about 25nm, although this range can vary widely. The average diameter of any given preparation can be readily determined by quasi-elastic light scattering techniques.

For more effective solubilization of some cytokines, for example, their point modification with fatty acid residues that act as hydrophobic anchors during incorporation of such agents into block copolymer micelles is required. For some cytokines, the incorporation into the micelles formed by the block-copolymer is achieved through the covalent conjugation of the cytokine and block copolymer. Various methods of such conjugation are used. These include cross-linking of the drug directly to an activated terminal group of a block copolymer of conjugation via a spacer groups using various heterobifunctional agents.

A number of pluronics are designed to meet the following formula:



(IX)

Of course, the ordinarily skilled artisan will recognize that the values of m and n will usually represent a statistical average and that the number of repeating units of the first block of a given molecule will generally not be exactly the

number of repeating units of the third block. The characteristics of a number of pluronics, described with reference to formula (IX), are as follows:

Copolymer	Hydrophobe weight	CMC (% w/v)	Hydrophobe percentage
Pluronic L61	1750	0.0003	90
Pluronic L64	1750	0.002	60
Pluronic F68	1750	4-5	20
Pluronic P85	2250	0.005 - 0.007	50
Pluronic Fl27	4000	0.003 - 0.005	30
Pluronic F108	3250	0.0035 - 0.007	20

These CMC values were determined by the surface tension method described in Kabanov *et al.*, *Macromolecules* 28: 2303-2314 (1995).

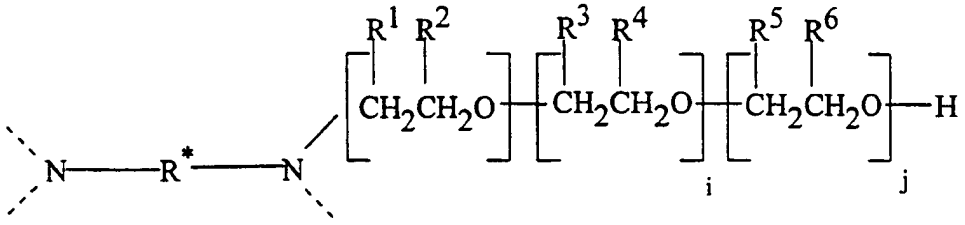
Additional specific poly(oxyethylene)-poly(oxypropylene) block copolymers relevant to the invention include:

Pluronic	Hydrophobe Weight	Hydrophobe Percentage
L31	950	90%
F35	950	50%
L42	1200	80%
L43	1200	70%
L44	1200	60%
L61	1750	90%
L62	1750	80%
L63	1750	70%
L64	1750	60%
P65	1750	50%
F68	1750	20%
P75	2050	50%
L81	2250	90%
P84	2250	60%
P85	2250	50%
F87	2250	30%
F88	2250	20%
L92	2750	80%
F98	2750	20%

P104	3250	60%
P105	3250	50%
F108	3250	20%
L121	4000	90%
L122	4000	80%
L123	4000	70%
F127	4000	30%
10R5	1000	50%
10R8	1000	20%
12R3	1200	70%
17R2	1700	80%
Pluronic (cont'd)	Hydrophobe Weight	Hydrophobe Percentage
17R1	1700	90%
17R2	1700	80%
17R4	1700	60%
17R8	1700	20%
22R4	2200	60%
25R1	2500	90%
25R2	2500	80%
25R4	2500	60%
25R5	2500	50%
25R8	2500	50%
31R1	3100	90%
31R2	3100	80%
31R4	3100	60%

*All copolymers above this conform to formula (IX), this copolymer and those below conform to formula (VII).

The diamine-linked pluronic of formula (VIII) can also be a member of the family of diamine-linked polyoxyethylene-polyoxypropylene polymers of formula:



(X)

wherein the dashed lines represent symmetrical copies of the polyether extending off the second nitrogen, R* an alkylene of about 2 to about 6 carbons, a cycloalkylene of about 5 to about 8 carbons or phenylene, for R^1 and R^2, either (a) both are hydrogen or (b) one is hydrogen and the other is methyl, for R^3 and R^4 either (a) both are hydrogen or (b) one is hydrogen and the other is methyl, if both of R^3 and R^4 are hydrogen, then one R^5 and R^6 is hydrogen and the other is methyl, and if one of R^3 and R^4 is methyl, then both of R^5 and R^6 are hydrogen. The $-NH_2-CH_2CH_2-NH_2-$ group of formula (VIII) and the N-R*-N group of formula (X) are examples of linking groups, *L*, of formula (IV).

Those of ordinary skill in the art will recognize that even when the practice of the invention is confined for example, to poly(oxyethylene)-poly(oxypropylene) compounds, the above exemplary formulas are too confining. An important feature is that the average Hansch-Leo fragmental constant of the monomers in an A-type block be about -0.4 or less. Thus, the units making up the first block need not consist solely of ethylene oxide. Similarly, not all of the B-type block need consist solely of propylene oxide units. Instead, the blocks can incorporate monomers other than those defined in formulas (V)-(X), so long as the parameters of the first embodiment are maintained. Thus, in the simplest of examples, at least one of the monomers in block A might be substituted with a side chain group as previously described.

In another aspect, the invention relates to a drug composition made up of a block copolymer at least one of formulas (I)-(X), wherein the A-type and B-type blocks are substantially made up of repeating units of formula $-O-R^5$, where R^5 is:

(1) $-(CH_2)_n-CH(R^6)-$, wherein n is zero or an integer from about 1 to about 5 and R^6 is hydrogen, cycloalkyl having about 3 to about 8 carbon atoms, alkyl having about 1 to about 6 carbon atoms, phenyl, alkylphenyl wherein the alkyl has about 1 to about 6 carbon atoms, hydroxy, hydroxyalkyl, wherein the alkyl has about 1 to about 6 carbon atoms, alkoxy having about 1 to about 6 carbon atoms, an alkyl carbonyl having about 2 to about 7 carbon atoms, alkoxycarbonyl, wherein the alkoxy has about 1 to about 6 carbon atoms, alkoxycarbonylalkyl,

wherein the alkoxy and alkyl each independently has about 1 to about 6 carbon atoms, alkylcarboxyalkyl, wherein each alkyl independently has about 1 to about 6 carbon atoms, aminoalkyl wherein the alkyl has about 1 to about 6 carbon atoms, alkylamine or dialkylamino, wherein each alkyl independently has about 1 to about 6 carbon atoms, mono- or di-alkylaminoalkyl wherein each alkyl independently has about 1 to about 6 carbon atoms, chloro, chloroalkyl wherein the alkyl has from about 1 to about 6 carbon atoms, fluoro, fluoroalkyl wherein the alkyl has from about 1 to about 6 carbon atoms, cyano or cyano alkyl wherein the alkyl has from about 1 to about 6 carbon atoms or carboxyl;

(2) a carbocyclic group having about 3 to about 8 ring carbon atoms, wherein the group can be for example, cycloalkyl or aromatic groups, and which can include alkyl having about 1 to about 6 carbon atoms, alkoxy having about 1 to about 6 carbon atoms, alkylamino having about 1 to about 6 carbon atoms, dialkylamino wherein each alkyl independently has about 1 to about 6 carbon atoms, amino, sulfonyl, hydroxy, carboxyl, fluoro or chloro substitutions, or

(3) a heterocyclic group, having about 3 to about 8 ring atoms, which can include heterocycloalkyl or heteroaromatic groups, which can include from about 1 to about 4 heteroatoms selected from the group consisting of oxygen, nitrogen, sulfur and mixtures thereto, and which can include alkyl having about 1 to about 6 carbon atoms, alkoxy having about 1 to about 6 carbon atoms, alkylamino having about 1 to about 6 carbon atoms, dialkylamino wherein each alkyl independently has about 1 to about 6 carbon atoms, amino, sulfonyl, hydroxy, carboxyl, fluoro or chloro substitutions.

Preferably, n is an integer from about 1 to about 3. The carbocyclic or heterocyclic groups comprising R^5 preferably have from about 4 to about 7 ring atoms, more preferably about 5 about 6. Heterocycles preferably include from about 1 to about 2 heteroatoms, more preferably, the heterocycles have one heteroatom. Preferably, the heterocycle is a carbohydrate or carbohydrate analog.

Those of ordinary skill will recognize that the monomers required to make these polymers are synthetically available. See, Vaughn *et al.*, *J. Am. Oil Chem.*

Soc., 28: 294 (1951). In some cases, polymerization of the monomers will require the use of suitable protective groups, as will be recognized by those of ordinary skill in the art. Generally, the A and B-type blocks are at least about 80% comprised of $-OR^5-$ repeating units, more preferably at least about 90%, yet more preferably at least about 95%.

In another aspect, the invention relates to a drug composition made up of a block copolymer of one of formulas (I)-(X) wherein the A-type and B-type blocks consist essentially of repeating units of formula $-0-R^7$, wherein R^7 is a C_1 to C_6 alkylene group.

The Hansch-Leo estimate of the octanol-water partitioning coefficient (P) for an organic molecule is calculated by the following formula:

$$\text{Log } P = a_n f_n + \Sigma b_m F_m$$

where the f_n values are the fragmental constants for the different groups in the molecule, the an values are the number of any type of group in the molecule, the FM values are factors for certain molecular features such as single bonds or double bonds, and the bm values are the number of any such molecular feature. For instance, the Hansch-Leo fragmental constant for an ethylene oxide repeating unit ($-CH_2CHO-$) would be:

$$2f_c + 4f_H + f_0 + (4-1)F_b = 2(0.20) + 4(0.23) + (-1.82) + 3(-0.12) = -0.86$$

The Hansch-Leo fragmental constant for a propylene oxide ($-CH_2CH(CH_3)O-$) repeating unit would be:

$$2f_c + f_{CH3} + 3fH + f_o + (4-1)F_b = 2(0.2) + 0.89 + 3(0.23) + (-1.82) + 3(-O.12) = -0.2$$

Those of ordinary skill in the art will recognize that the Hansch-Leo approach to estimating partition constants, in which approach the Hansch-Leo fragmental constants are applied, does not yield precisely the empirical partition constant. See Hansch and Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, *Wiley*, New York, 1979; James, *Solubility and Related Properties*,

Marcel Dekker, New York, 1986, pp. 320-325. However, the approach is precise enough to define the hydrophobicity features of the polymeric delivery vehicle.

The block copolymers utilized in the invention will preferably form micelles in isotonic aqueous solutions at a physiological temperature having diameter from about 10nm to about 100nm. Micelles are supramolecular complexes of certain amphiphilic molecules that form in aqueous solutions due to microphase separation of the nonpolar portions of the amphiphiles. Micelles form when the concentration of the amphiphile reaches, for a given temperature, a CMC that is characteristic of the amphiphile. By varying the sizes of the hydrophilic and hydrophobic segments of the block copolymers, the tendency of the copolymers to form micelles at physiological conditions, as well as the average size of the micelles formed at physiological conditions, can be varied. These tendencies can also be adjusted by blending copolymers with differing mixes of hydrophobic and hydrophilic blocks. The micelles have a dense core formed by the water insoluble repeating units of the B blocks and lipophilic portions of a biological agent dissolved therein, and a hydrophilic shell formed by the A blocks and hydrophobic portions of the biological agent. The micelles have translational and rotational freedom in aqueous environment, and aqueous environments containing the micelles have low viscosity similar to water. Micelle formation typically occurs at copolymer concentrations from about 0.001 to 5% (w/v).

The small size of the micelles formed by block copolymers of the invention is believed to allow these micelles to penetrate in small capillaries and to be taken up by cells. The micelles also can incorporate large amounts of appropriate biological agents. For instance, micelles formed by Pluronic L61 can incorporate at least 1 mg of doxorubicin per 2 mg of copolymer.

The effective retention of a drug within the micelles of the invention can be quantified in terms of the partitioning coefficient (P) determined using formula:

$$P = [Agent]_m / [Agent]aq$$

where $(Agent]_{aq}$ is the concentration of biological agent in an aqueous environment outside of the micelles and $[Agent]_m$ is the concentration of agent in the micelles. In some cases, P is easily and accurately estimated based on the difference fluorescence properties of certain agents when in an aqueous vs. a more hydrophobic environment.

A minor portion of a targeting molecule made up of a targeting moiety coupled to a lipophilic moiety comprising a hydrocarbon having from about 3 to about 41 carbon atoms is incorporated into the micelles of the compositions of the targeting embodiment of the invention. This portion typically comprises no more than about 10% w/w of the copolymer components of a composition. The lipophilic moieties are believed to act as hydrophobic "anchors", which are incorporated non-covalently into the block-copolymer micelles so that the targeting moiety becomes part of, but extends beyond, the micelle. Such targeting moieties are preferably also incorporated into the micelles used in the brain chemotherapy embodiment of the invention. However, for the brain chemotherapy embodiment the lipophilic moiety can be any lipophilic moiety effective to non-covalently associate the targeting moiety with the micelles. For the brain chemotherapy embodiment, the lipophilic moiety can be, for example a fatty acid residue, a lipid, phospholipid, or a natural or synthetic polymer. Because of availability and ease of use, lipophilic moieties containing hydrocarbon groups such as fatty acid residues are preferred.

The targeting moieties have affinity for a cellular, tissue, viral or substratum site. Typical targeting moieties include without limitation antibodies and hormones with affinity for a cellular binding component, any molecule containing a carbohydrate moiety recognized by a cellular binding component and drugs that bind to a cellular binding component. The phrase "binding component" includes both receptor and acceptor molecules. Preferably, the binding component is a cell-surface binding component. Both polyclonal and monoclonal antibodies which are either available commercially or described in the literature can be employed. Alternatively the ligand can be a naturally occurring protein, such as insulin, that binds to a target site. A non-limiting example of a targeting moiety is

the anti-α_2-GP antibody to brain glial cells (α_2-glycoprotein) which is described by Slepnev *et al.*, *Bioconjugate Chem., 3:* 273-274 (1992).

To retain as much of the specificity of the polypeptide, preferably only one or two lipophilic moieties are bound to each polypeptide molecule. This binding can be achieved by the method described by Kabanov *et al., Protein Engineering,* 3, 39-42 (1989), the contents of which are incorporated herein by reference. In this method the lipophilic moiety or a reactive analog thereof is reacted with the targeting moiety in the presence of the surfactant sodium bis(2-ethylhexyl)sulfosuccinate {AOT®}, octane and a small amount of water will form reversed micelles, that is micelles with water on the inside and octane on the outside. These reversed micelles serve as microreactors allowing uniform point modification of the polypeptide molecules with lipophilic moieties. Reactive derivatives of fatty acids such as stearoyl chloride or lauroyl chloride can be reacted with polypeptides or other hydrophilic targeting moieties using this reaction system. Because the reaction system allows for the level of fatty acyl substitution to be limited, greater biological activity and solubility of the targeting moiety is generally preserved.

The pharmaceutical compositions of the invention can be administered by a number of routes, including without limitation orally, topically, rectally, vaginally, by pulmonary route, for instance, by use of an aerosol, or parenterally, including but not limited to intramuscularly, subcutaneously, intraperitoneally, intra-arterially or intravenously. The compositions can be administered alone, or can be combined with a pharmaceutically-acceptable carrier or excipient according to standard pharmaceutical practice. For oral administration, the compositions can be used in the form of tablets capsules, lozenges, troches, powders, syrups, elixirs, aqueous solutions and suspensions, and the like. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use,

the compositions can be combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring agents can be added. For parenteral administration, sterile solutions of the conjugate are usually prepared, and the pHs of the solutions are suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic. For ocular administration, ointments or droppable liquids may be delivered by ocular delivery systems known to the art such as applicators or eye droppers. Such compositions can include mucomimetics such as hyaluronic acid, chondroitin sulfate, hydroxypropyl methylcellulose or poly(vinyl alcohol), preservatives such as sorbic acid, EDTA or benzylchronium chloride, and the usual quantities of diluents and/or carriers. For pulmonary administration, diluents and/or carriers will be selected to be appropriate to allow the formation of an aerosol.

Suppository forms of the compositions of the invention are useful for vaginal, urethral and rectal administrations. Such suppositories will generally be constructed of a mixture of substances that is solid at room temperature but melts at body temperature. The substances commonly used to create such vehicles include theobroma oil, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycol of various molecular weights and fatty acid esters of polyethylene glycol. *See* Remington's Pharmaceutical Sciences, 16th Ed., Mack Publishing, Easton, PA, 1980, pp. 1530-1533 for further discussion of suppository dosage forms. Analogous gels or creams can be used for vaginal, urethral and rectal administrations.

A variety of biological agents are suitable for use in the invention. This includes, without limitation, proteins, peptides or derivatives thereof (*e.g.*, polypeptides) including cytokines, hormones (such as insulin), and the like, recombinant soluble receptors, monoclonal antibodies, human growth hormones, tissue plasminogen activators, clotting factors, vaccines, colony stimulating factors, erythropoietins, enzymes, and dismultase.

Where proteins, peptides or derivatives thereof are to be used, the protein, peptide or derivative thereof of choice (which may include a mixture of several of these) is preferably either covalently modified with a hydrophobic substituent (*e.g.*, a fatty acid or lipid residue), or incorporated into a micelle of a block copolymer of poly(oxyethylene)-poly(oxypropylene) (POE-POP) in an aqueous dispersion, or covalently modified with a hydrophobic substituent, and then incorporated into a micelle of a block-copolymer of poly(oxyethylene)-poly(oxypropylene) as described herein.

Incorporation of proteins, peptides, or derivatives thereof into block copolymer micelles is performed either noncovalently by solubilization of the protein, peptide or derivative thereof in block copolymer aqueous solution, or covalently by cytokine conjugation with the block-copolymer and subsequent solubilization of the obtained conjugate in the block copolymer aqueous solution.

Without wishing to be bound to a specific theory it is further believed that modification of the protein, peptide, or derivative thereof with a hydrophobic substituent generally improves the biological activity of the protein, peptide, or derivative thereof, while mixture with the block copolymers of the composition the invention provide for increased stability, improved transport and decreased side effects of such modified proteins, peptide or derivatives thereof. The hydrophobes useful in the context of this embodiment include, but are not limited to, fatty acids and derivatives thereof, fatty acid soaps including salts of saturated and unsaturated fatty acids and derivatives (*e.g.*, adrenic acid, arachidonic acid, 2-octenoic acid, octanoic acid, nonanoic acid, decanoic acid, undecanoic acid, undecelenic acid, lauric acid, myristoleic acid, myristic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, nonanedecanoic acid, henelcosanoic acid, docasanoic acid, tricosanoic acid, tetracosanoic acid, *cis*-15-tetracosenoic acid, hexacosanoic acid, heptacosanoic acid, octecosanoic acid, triocantanoic acid), salts of hydroxy-, hydroperoxy-, polyhydroxy-, epoxy-fatty acids (see for example, Ingram and Brash, *Lipids*, 1988, 23:340; Honn *et al.*, *Prostaglandins*, 1992, 44:413; Yamamoto, Free Radic, *Biol. Med.*, 1991, 10:149; Fitzpatrick and Murphy, *Pharmacol Rev.*, 1989, 40:229; Muller *et al.*,

Prostaglandins, 1989, 38:635; Falgueyret *et al.*, *FEBS Lett.*, 1990, 262:197; Cayman Chemical Co., 1994 Catalog, pp. 78-108), residues of carboxylic acids (*e.g.*, valeric acid, trans-2,4-pentadionoic acid, hexanoic acid, *trans*-2-hexenoic acid, *trans*-3-hexenoic acid, 2,6-heptadienoic acid, 6-heptenoic acid, heptanoic acid, pimelic acid, suberic acid, sebacicic acid, azelaic acid, undecanedioic acid, decanedicarboxylic acid, undecanedicarboxylic acid, dodecanedicarboxylic acid, hexadecanedioic acid, docasenedioic acid, tetracosanedioic acid, prostanoic acid and its derivatives (*e.g.*, Prostaglandins) (see, for example, Nelson *et al.*, *C&EN* 1982, 30-44; Frolich, *Prostaglandins*, 1984, 27:349; Cayman Chemical Co., 1994 Catalog, pp. 26-61), leukotrienes and lipoxines (see for example, Samuelsson *et al.*, *Science*, 1987, 237:1171; Cayman Chemical Co., 1994 Catalog, pp. 64-76), alkyl phosphates, O-phosphates (*e.g.*, benfotiamine), alkyl phosphonates. natural and synthetic lipids (*e.g.*, dimethylallyl pyrophosphate-ammonium salt, S-fernesylthioacetic acid, farnesyl pyrophphosphate, 2-hydroxymyristic acid, 2-fluoropalmitic acid, inositoltriphosphates, geranyl pyrophosphate, geranygeranyl pyrophosphate, α-hydroxyfarnesyl phosphonic acid, isopentyl pyrophoshate, phosphatidylsorines, cardiolipines, phosphatidic acid and derivatives, lysophosphatidic acids, sphingolipids and the like), synthetic analogs of lipids such as sodium-dialkyl sulfosuccinate (*e.g.*, Aerosol OT®), n-alkyl ethoxylated sulfates, n-alkyl monothiocarbonates, alkyl- and aryisulfates (asaprol, azosulfamide, *p*-(benzyl-sulfonamideo)benzoic acid, cefonicid, CHAPS), mono- and dialkyl dithiophosphates, N-alkanoyl-N-methylglucamine, perfluoroalcanoate, cholate and desoxychoate salts of bile acids, 4-chloroindoleacetic acid, cucurbic acid, jasmonic acid, 7-epi jasmonic acid, 12-oxo-phytodienoic acid, traumatic acid, tuberonic acid, abscisic acid, acitertin, and the like.

The hydrophobe useful in this invention is also produced by long alkyl chain amines including primary, secondary and tertiary amines (*e.g.*, hexylamine. heptylamine, octylamine, decylamine, undecylamine, dodecylamine, pentadecyl amine, hexadecyl amine, oleylamine, stearylamine, diaminopropane, diaminobutane, diaminopentane, diaminohexane, diaminoheptane, diaminooctane, diaminononane, diaminodecane, diaminododecanery amines, N,N-distearylamine,

N,N',N'-polyoxyethylene(10)-N-tallow-1,3-diaminopropane) and quaternary amine salts (*e.g.*, dodecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, alkyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, benzalkonium chloride, benzethonium chloride, benzylonium bromide, benzyldimethyidodecylammonium chloride, benzyldimethylhexadecylammonium chloride, benzyltrimethylammonium methoxide, cetyldimethylethylammonium bromide, dimethyldioctadecyl ammonium bromide, methylbonzethonium chloride, decamethonium chloride, methyl mixed trialkyl ammonium chloride, methyl trioctylammonium chloride), 1,2-diacyl-3-(trimethylammonio)propane (acyl groupn = dimyristoyl, dipalmitoyl, distearoyl, dioleoyl), 1,2-diacy-3-(dimethylammonio)propane (acyl group = dimyristoyl, dipalmitoyl, distearoyl, dioleoyl), 1,2-dioleoyl-3-(4'-trimethylammonio) butanoyl-sn-glycerol, 1,2-dioleoyl-3-succinyl-*sn*-glycerol choline ester, cholesteryl (4'-trimethylammonio) butanoate), heterocyclic amines, imidazoles, thiazolium salts, N-alkyl pyridinium and quinaldinium salts (*e.g.*, cetylpyridinium halide), N-alkylpiperidinium saits, dialkyldimethylammonium salts, dicationic bolaform electrolytes ($C_{l2}Me_6$; $C_{12}Bu_6$), dialkylglycetylphosphorylcholine, lysolecithin), cholesterol hemisuccinate choline ester, lipopolyamines (*e.g.*, dioctadecylamidoglycylspermlne (DOGS), dipalmitoyl phosphatidylethanolamidospermine (DPPES), N'-octadecylsperminecarboxamide hydroxytrifluoroacetate, N',N''-dioctadecylsperminecarboxamide hydroxytrlfluoroacetate, N'-nonafluoropentadecylo-sperminecarboxamide hydroxytrifluoroacetate, N',N''-dioctyl(sperminecarbonyl)glycinamide hydroxytrifluoroacetate, N'-(heptadecafluorodecyl)-N'-(nonafluoropentadecyl)-spermine-carbonyl)glycinamede hydroxytrifluoroacatate, N'-[3,6,9-trioxa-7-(2'-oxaeicos-11'-enyl)-heptaeicos-18enyl]sperminecarboxamide hydroxytrifluoroacetate, N'-(1,2-dioleoyl-*sn*-glycero-3-phosphoethanoyl)spermine carboxamide hydroxytrifluoroacetate) (see, for example, Behr *et al.*, *Proc. Natl. Acad. Sci.*, 1989, 86:6982; Remy *et al.*, *Bioconjugate Chem.*, *1994,* 5:647), 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA) (see, for example, Ciccarone *et al.*, *Focus* 1993, 15:80), N,N',N'', N'''-tetramethylN,N',N'',N'''-

tetrapalmitylspermine (TM-TPS) (Lukow *et al., J. Virol.*, 1993, 67:4566), N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylamonium chloride (DOTMA) (see, for example, Feigner, *et al, Proc. Natl. Acad. Sci.*, USA, 1987, 84:7413; Ciccarone *et al, Focus*, 1993, 15:80), dimethyl dioctadecylammonium bromide (DDAB) (see, for example, Whitt *et al., Focus*, 1991, 13:8), 1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium bromide (DORI) (see, for example, Feigner *et al, J. Biol. Chem.*, 1994, 269:2550), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE), (see, for example, Feigner *et al., J. Biol. Chem.*, 1994, 269:2550), 1,2-dioleyloxypropyl-3-dimethyl-hydroxypropyl ammonium bromide (DORIE-HPe) (see, for example, Feigner *et al., J. Biol. Chem.*. 1994, 269:2550), 1,2-dioleyloxypropyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB) (see, for example, *Felgner et al., J. Biol., Chem.* 1994,269:2560), 1,2-dioleyloxypropyl-3-dimethylhydroxypentyl ammonium bromide (DORIE-HPe) (see for example, *Feigner et al., J. Biol. Chem.*, 1994, 269:2550), 1,2-dimyristyloxypropyl3-dimethyl-hydroxyethyl ammonium bromide (DMRIE) (see for example, *Feigner et al., J. Biol. Chem*, 1994, 269:2550), 1,2--dipalmitoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DPRIE) (see, for example, *Feigner et al., J. Biol. Chem.*, 1994, 269:2550), 1,2-distearoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DSRIE) (see, for example, *Feigner et al., J. Biol. Chem.*, 1994, 269;2550), N,N-dimethyl-N-[2-(2-methyl-4-(1,1,3,3tetramethylbutyl)-phenoxy]ethoxy)ethyl]-benzenemethanaminium chloride (DEBDA), N-[1-(2,3-dioleyloxy)propyl]-N,N,N,-trimethylammonium methylsulfate (DOTAB), lipopoly-L(or D)-lysine (see, for example, Zhou, *et al., Biochim. Siophys. Acta.*, 1991, 1065:8), poly(L or D)-lysine conjugated to N-glutarylphosphatidylethanolamine lysine (see, for example, Zhou, *et al., Biochim. Biophys. Acta*, 1991, 8:1065), didodecyl glutamate ester with pendent amino group ($C_{12}GluPhC_nN^+$) (see, for example, Behr, *Bioconjugate Chem.*, 1994, 5:382), ditetradecyl glutamate ester with pendant amino group ($C_{14}GluC_nN^+$) (see, for example, Behr, *Bioconjugate Chem.*, 1994, 5:382), 9-(N',N''-dioctadecylglycinamido)acridine (see, for example, Remy et *al., Bioconjugate Chem.*, 1994, 5:647), ethyl 4-[[N-[3-bis(octadecylcarbamoyl)-2-oxapropyl-carbonyl]glycinamido]pyrrole-2-carboxamido]-4-pyrrole-2-

carboxylate (see, for example, Remy *et al.*, *Bioconjugate Chem.*, 1994, 5:647), N',N'-dioctadecylomithylglycinamide hydro-ptrifluoroacetate (see, for example, Remy *et al.*, *Bioconjugate Chem.*, 1994, 6:647), cationic derivatives of cholesterol (*e.g.*, cholesteryl-3β-oxysuccinamido-ethylene-trimethylammonium salt, cholesteryl-3β-oxysuccinamidoethylenedimethylamine, cholesteryl-3β-carboxyamidoethylone-trimethylammonium salt, cholesteryl-3β-carboxyamido-ethylenedimethyl-amine, 3-β-[N-(N',N'-dimethylaminoetane-carbomoyl] cholesterol) (see, for example, Singhal and Huang, *Gene Therapeutics*, Wolff, Ed., p.118 *et seq.*, Birkhauser, Boston, 1993), pH sensitive cationic lipids (*e.g.*, 4-(2,3-bis-palmitoyloxy-propyl)-1-methyl-1H-imidazole, 4-(2,3-bis-oleoyloxy-propyl)-l-methyl-1H-imidazole, cholesterol-(3-imidazol-1-yl-propyl) carbamate, 2,3-bis-palmitoylpropyl-pyridin-4-yl-amine) and the like (see, for example, Budker, *et al.*, *Nature Biotechnology*, 1996, 14:760).

The hydrophobes that can be used in this invention with the protein, peptide or derivative thereof also include residues of fluorocarbons and mixed fluorocarbon-hydrocarbon surfactants. See for example, Mukerjoe, *P. Coll. Surfaces A: Physkochem. Engin. Asp.*, 1994, 84: 1; Guo *et al.*, *J. Phys. Chem.*, 1991, 95:1829, Guo *et al.*, *J. Phys. Chem.*, 1992, 96:10068. Surfactants that are useful in current inventions includes, but is not limited to, the salts of perfluorocarboxylic acids (*e.g.*, pentafluoropropionic acid, heptafluorobutyric acid, nonanfluoropentanoic acid, tridecafluoroheptanoic acid, pentadecafluorooctanoic acid, heptadecafluorononanoic acid, nonadecafluorodecanoic acid, perfluorododecanoic acid, perfluorotetradecanoic acid, hexafluoroglutaric acid, perfluoroadipic acid, perfluorosuberic acid, perfluorosebacicic acid), double tail hybrid surfactants $(C_mF_{2m+l})(C_nH_{2n+1})CH\text{-}OSO_3Na$ (see, for example, Guo *et al.*, *J. Phys. Chem.*, 1992, 96:6738, Guo *et al.*, *J. Phys. Chem.*, 1992, 96:10068; Guo *et al.*, *J. Phys. Chem.*, 1992, 96:10068), fluoroallphatic phosphonates, fluoroaliphatic sulphates, and the like.

The protein, peptide or derivative thereof may also be modified with derivatives of nonionic or zwitterionic surfactants including but not limited to phosholipids (*e.g.*, phosphatidylethanolamines, phosphatidylglycerols,

phosphatidylinositols, diacyl phosphati-dyicholines, di-O-alkyl phosphatidylcholines, platelet-activating factors, PAF agonists and PAF antagonists, lysophosphatidylcholines, lysophosphatidylethanolamines, lysophosphatidylglycerols, lysophosphatidylinositols, lysoplatelet-activating factors and analogs, and the like), saturated and unsaturated fatty acid derivatives (*e.g.*, ethyl esters, propyl esters, cholesteryl esters, coenzyme A esters, nitrophenyl esters, naphthyl esters, monoglycerides, diglycerides, and triglycerides, fatty alcohols, fatty alcohol acetates, and the like), lipopolysaccharides, glyco- and shpingolipids (*e.g.*, ceramides, cerebrosides, galactosyldiglycerides, gangilosides, lactocerebrosides, lysosulfatides, psychosines, shpingomyelins, sphingosines, sulfatides), chromophoric lipids (neutral lipids, phospholipids, cerebrosides, sphingomyelins), cholesterol and cholesterol derivatives, Amphotericin B, abamectin, acediasulfone, n-alkylphenyl polyoxyethylene ether, n-alkyl polyoxy-ethylene ethers (*e.g.*, Triton™), sorbitan esters (*e.g.*, Span™), polyglycol ether surfactants (Tergitol™), polyoxyethylenesorbitan (*e.g.*, Tween™), polysorbates, polyoxyethylated glycol monoethers (*e.g.*, Brij™, polyoxylethylene 9 lauryl ether, polyoxylethylene 10 ether, polyoxylethylene 10 tridecyl ether), lubrol, copolymers of ethylene oxide and propylene oxide (*e.g.*, Pluronic™, Pluronic-R™, Tetronic™, Pluradot™), alkyl aryl polyether alcohol (Tyloxapol™), perfluoroalkyl polyoxylated amides, N,N-bis[3-D-gluconamidopropyl]cholamide, decanoyl-N-methylglucamide, *n*-decyl α-D-glucopyranozide, n-decyl β-D-glucopyranozide, n-decyl β-D-maltopyranozide, n-dodecyl β-D-glucopyranozide, n-undecyl β-D-glucopyranozide, n-heptyl β-D-glucopyranozide, n-heptyl β-D-thioglucopyranozide, n-hexyl β-D-glucopyranozide, n-nonanoyl β-D-glucopyranozide 1-monooleyl-racglycerol, nonanoyl-N-mothylglucamide, n-dodecyl α-D-Maltoside, n-dodecyl β-D-maltoside, N,N-bis[3-gluconamidepropyl]deoxycholamide, diethylone glycol monopentyl ether, digitonin, heptanoyl-N-methylglucamide, heptanoyl-N-methylglucamide, octanoyl-N-methylglucamide, n-octyl β-D-glucopyranozide, n-octyl β-D-glucopyranozide, n-octyl β-D-thiogalactopyranozide, n-octyl β-D--thioglucopyranozide, betaine ($R_1R_2R_3N^+R'CO_2^-$, where $R_1R_2R_3R'$ are hydro-carbon chains), sulfobetaine ($R_1R_2R_3N^+R'SO_3^-$), phoshoplipids (*e.g.*, dialkyl

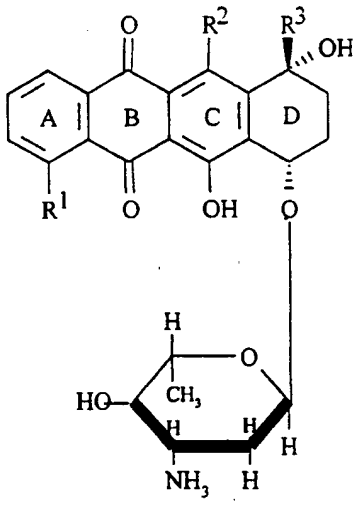
phosphatidylcholine), 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-l-propanesulfonate, 3-[(3-cholamidopropyl)-dimethylammonio]-l-propanesulfonate, N-decyl-N,N-dimethyl-3-ammonio-l-propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-l-propanesulfonate, N-hexadecyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate, N-octadecyl-N,N-dimethyl-3-ammonio-l-propanesulfonate, N-octyl-N,N-dimethyl-3-ammonio-l-propanesulfonate, N-tetradecyl-N,N-dimethyl-3-ammonio-l-propanesulfonate, dialkyl phosphatitidylethanolamine.

Both a cytokine covalent modification with a hydrophobic substituent and incorporation of a cytokine (either unmodified or modified with a hydrophobic group into a block copolymer micelle) lead to enhancement of specific immunomodulatory activity of this cytokine, and reduction of its side effects on the patient. These effects result from: {i} the increase of apparent affinity of a modified or micelle-incorporated cytokine to receptor-bearing (target) cells, {ii} increase of the efficacy of the cytokine penetration into the target cells, and {iii} decrease of cytokine nonspecific interactions with organs and tissues other than those providing its immunomodulatory effect.

A variety of human and animal cytokines are suitable for use in the present compositions. These include interferons, interleukins, tumor necrosis factors (TNFs) such as TNFα, and a number of other protein and peptide factors controlling functions of the immune system. It will be appreciated that this extends to mixtures of several such agents, and the invention is not directed to the underlying specific activity of the cytokines themselves, but rather to the compositions themselves.

Cytokine covalent modification with a hydrophobic substituent can be performed in reversed micelles of AOT® in octane that serve as microreactors allowing uniform point modification of peptide or protein molecules with fatty acid or lipid residues (1 to 5 residues per protein or peptide molecule). This makes it possible to preserve the water solubility and biological activity of modified agents. Kabanov, *et al.*, *Protein Engineering*, 3(1), 39-42 (1989).

Chemotherapeutic agents appropriate for use in this invention include, without limitation, vinca alkaloids such as vincristine and vinblastine, mitomycin-type antibiotics such as mitomycin C and N-methyl mitomycin C, bleomycin-type antibiotics such as bleomycin A2, antifolates such as methotrexate, aminopterin, and dideaza-tetrahydrofolic acid, colchicine, demecoline, etoposide, taxanes such as paclitaxel (Taxol®), anthracycline antibiotics and others. The anthracycline antibiotics exemplify drugs having delivery problems due to low stability, the development of drug resistance in the target tissue, or rapid metabolism. These antibiotics typically include a fused tetracycline aglycone ring system joined at the 7-position to daunosamine. They include, for instance, the compounds represented by the formula:



wherein R^1 is hydroxy or methoxy; R^2 is hydrogen or hydroxy; and R^3 is ethyl, acetyl, hydroxyacetyl, or an ester of hydroxyacetyl. These tetracycline antibiotics, like many anti-neoplastic agents, are believed to act by intercalating between the planar aromatic ring structures of DNA, thereby interfering with DNA replication. See, Neidle and Waring, *Molecular Aspects of Anti-Cancer Drug Action,* Pitman Press (1983). Neoplastic cells are generally particularly susceptible, since they are actively replicating and thus synthesizing replica copies of their DNA. Such tetracycline antibiotics include, without limitation, doxorubicin, daunorubicin, carminomycin, epirubicin, idarubicin, mithoxanthrone, 4-demethoxy-daunomycin, 11-deoxydaunorubicin, 13-deoxydaunorubicin,

adriamycin-14-benzoate, adriamycin-14-octanoate, or adriamycin-14-naphthaleneacetate.

Preferred classes of biological agents (including chemotherapeutic agents) include anti-neoplastic agents, antibacterial agents, antiparasitic agents, anti-fungal agents, CNS agents, immunomodulators and cytokines, toxins and neuropeptides. Biological agents for which target cells tend to develop resistance mechanisms are also preferred. Particularly preferred biological agents include anthracyclines such as doxorubicin, daunorubicin, epirubicin, idarubicin, mithoxanthrone or carminomycin, vinca alkaloids, mitomycin-type antibiotics, bleomycin-type antibiotics, azole antifungals such as fluconazole, polyene antifungals such as amphotericin B, taxane-related antineoplastic agents such as paclitaxel and immunomodulators such as tumor necrosis factor alpha (TNF-α), interferons and cytokines.

Preferred biological agents (including chemotherapeutic agents) include without limitation additional antifungal agents such as amphotericin-B, flucytosine, ketoconazole, miconazole, itraconazole, griseofulvin, clotrimazole, econazole, terconazole, butoconazole, ciclopirox olamine, haloprogin, toinaftate, naftifine, nystatin, natamycin, undecylenic acid, benzoic acid, salicylic acid, propionic acid and caprylic acid. Such agents further include without limitation antiviral agents such as zidovudine, acyclovir, ganciclovir, vidarabine, idoxuridine, trifluridine, foxcarnet, amantadine, rimantadine and ribavirin. Such agents further include without limitation antibacterial agents such as penicillin-related compounds including 9-lactam antibiotics, broad spectrum penicillins and penicillinase-resistant penicillins (such as methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, amoxicillin, ampicillin, ampicillin-sulbactam, azocillin, bacampicillin, carbenicillin, carbenicillin indanyl, cyclacillin, mezlocillin, penicillin G, penicillin V, piperacillin, ticarcillin, imipenem and aztreonam), cephalosporins (cephalosporins include first generation cephalosporins such as cephapirin, cefaxolin, cephalexin, cephradine and cefadroxil; second generation cephalosporins such as cefamandole, cefoxitin, cefaclor, cefuroxime, cefuroxime axetil, cefonicid, cefotetan and ceforanide; third generation cephalosporins such as

cefotaxime, ceftizoxime, ceftriaxone, cefoperazone and ceftazidime), tetracyclines (such as demeclocytetracycline, doxycycline, methacycline, minocycline and oxytetracycline), beta-lactamase inhibitors (such as clavulanic acid), aminoglycosides (such as amikacin, gentamicin C, kanamycin A, neomycin B, netilmicin, streptomycin and tobramycin), chloramphenicol, erythromycin, clindamycin, spectinomycin, vancomycin, bacitracin, isoniazid, rifampin, ethambutol, aminosalicylic acid, pyrazinamide, ethionamide, cycloserine, dapsone, sulfoxone sodium, clofazimine, sulfonamides (such as sulfanilamide, sulfamethoxazole, sulfacetamide, sulfadiazine, and sulfisoxazole), trimethoprim-sulfamethoxazole, quinolones (such as nalidixic acid, cinoxacin, norfloxacin and ciprofloxacin), methenamine, nitrofurantoin and phenazopyridine. Such agents further include agents active against protozoal infections such as chloroquine, diloxanide furoate, emetine or dehydroemetine, 8-hydroxyquinolines, metronidazole, quinacrine, melarsoprol, nifurtimox, pentamidine, sodium stibogluconate and suramin.

A variety of central nervous system agents are suitable for use in the present composition. These include neuroleptics such as the phenothiazines (such as compazine, thorazine, promazine, chlorpromazine, acepromazine, aminopromazine, perazine, prochlorperazine, trifluoperazine, and thioproperazine), rauwolfia alkaloids (such as reserpine and deserpine), thioxanthenes (such as chlorprothixene and tiotixene), butyrophenones (such as haloperidol, moperone, trifluoperidol, timiperone, and droperidol), diphenylbutylpiperidines (such as pimozide), and benzamides (such as sulpiride and tiapride); tranquilizers such as glycerol derivatives(such as mephenesin and methocarbamol), propanediols (such as meprobamate), diphenylmethane derivatives (such as orphenadrine, benzotrapine, and hydroxyzine), and benzodiazepines(such as chlordiazepoxide and diazpam); hypnotics (such as zolpdem and butoctamide); 9-blockers (such as propranolol, acebutonol, metoprolol, and pindolol); antidepressants such as dibenzazepines (such as imipramine), dibenzocycloheptenes (such as amitriptyline), and the tetracyclics (such as mianserine); MAO inhibitors (such as phenelzine, iproniazide,and

selegeline); psychostimulants such as phenylethylamine derivatives (such as amphetamines, dexamphetamines, fenproporex, phentermine, amfepramone, and pemline) and dimethylaminoethanols (such as clofenciclan, cyprodenate, aminorex, and mazindol); GABA-mimetics (such as progabide), alkaloids (such as co-dergocrine, dihydroergocristine, and vincamine); cholinergics (such as citicoline and physosigmine); vasodilators (such as pentoxifyline); and cerebro active agents (such as pyritinol and meclofenoxate); as well as mixtures of several such agents.

Of particular interest are sedative-hypnotics such as the benzodiazepines, psycho-pharmacological agents such as the phenothiazines, thioxanthenes, butyrophenones, and dibenzoxazepines, and central nervous system stimulants. Since, the brain treatment embodiment of the invention is directed to compositions that improve the activity of biological agents, this embodiment of the invention can be applied to a wide variety of central nervous system agents by applying the principles and procedures described herein.

The compositions also can utilize a variety of polypeptides such as antibodies, toxins such as diphtheria toxin, peptide hormones, such as colony stimulating factor, and tumor necrosis factors, neuropeptides, growth hormone, erythropoietin, and thyroid hormone, lipoproteins such as α-lipoprotein, proteoglycans such as hyaluronic acid, glycoproteins such as gonadotropin hormone, immunomodulators or cytokines such as the interferons or interleukins, hormone receptors such as the estrogen receptor.

The block copolymers also can be used with enzyme inhibiting agents such as reverse transcriptase inhibitors, protease inhibitors, angiotensin converting enzymes, 5a-reductase, and the like. Typical of these agents are peptide and non-peptide structures such as finasteride, quinapril, ramipril, lisinopril, saquinavir, ritonavir, indinavir, nelfinavir, zidovudine, zalcitabine, allophenylnorstatine, kynostatin, delaviridine, *bis*-tetrahydrofuran ligands (see, for example Ghosh *et al.*, *J. Med. Chem.*, 39(17): 3278-90 (1966)), and didanosine. Such agents can be administered alone or in combination therapy; *e.g.*, a combination therapy

utilizing saquinavir, zalcitabine, and didanosine or saquinavir, zalcitabine, and zidovudine. See, for example, Collier *et al., Antiviral Res.,* 1996 Jam., 29(1): 99.

The dosage for a biological agent in a micellar composition will often be about that of the biological agent alone; dosages will be set by the prescribing medical professional considering many factors including the age, weight and condition of the patient and the pharmacokinetics of the agent. Often the amount of a micellar form of an agent required for effective treatment may be less than the amount required using the free biological agent. For daunorubicin use in treating cancer, a typical dosage will be about 1 mg per kg of body weight. Vinblastine is typically administered at a dose of from 0.1 to 0.2 mg per kg of body weight.

Generally, the biological agents used in the invention are administered to an animal in an effective amount. The effect of the copolymer used in the composition on effectiveness must be considered in determining effective amount. Generally, an effective amount is an amount effective to either (1) reduce the symptoms of the disease sought to be treated or (2) induce a pharmacological change relevant to treating the disease sought to be treated. For cancer, an effective amount includes an amount effective to: reduce the size of a tumor; slow the growth of a tumor; prevent or inhibit metastases; or increase the life expectancy of the affected animal.

In many cases, the metabolites of various biological agents create or enhance the unwanted effects resulting from administering the agent. This is certainly the case for anthracycline-based drugs, where metabolites are believed to lead to cardiotoxicity. See, Mushlin *et al., Br. J. Pharmacol.,* 110:975-982 (1993). The copolymer compositions of the invention can decrease the rate of metabolism for biological agents, thereby reducing the potential for harmful side effects.

Penetration of the brain by a biological agent can be measured by a number of techniques, as will be recognized by those of ordinary skill in the art. Such methods include isotope labeling, assessing animal behavior for the effects of a biological agent, and measuring lethal dosages for drugs with toxic effects that

occur at the brain. Such methods further include measuring decreases in the dosage required to elicit the appropriate biological response.

Various antifungal agents successfully treat human fungal infections. However, the therapeutic dose is often a compromise between achieving effective drug levels and avoiding toxic side effects. In recent years, the emergence of drug resistance among intrinsically sensitive species such as *Candida albicans* and the increasing incidence of intrinsically drug resistant species such as *Candida kruset* has prompted a search for newer antifungal agents.

Although fluconazole has a low incidence of side effects, the incidence of resistance is an increasing problem. Delivery vehicles that are effective in enhancing chemotherapeutic activity and reversing resistance to such agents is therefore desirable for this agent, as well as for other antimicrobial agents.

The following examples will serve to further typify the nature of the invention but should not be construed as a limitation on the scope thereof, which is defined solely by the appended claims.

EXAMPLE 1 - Micelle Size

Block copolymers of poly(oxyethylene)-poly(oxypropylene) having the ratios of poly(oxypropylene) to poly(oxyethylene) indicated below were dispersed in RPMI 1640 medium at the concentrations indicated below. The mixtures were incubated for 40 minutes at 300°C. The average micelle diameter was measured by quasielastic light scattering. See Kabanov *et al.*, *Macromolecules* 28: 2303-2314 (1995). The results were as follows:

Copolymer	Conc. (% w/v)	Avg. Diameter
F-68	1.0%	726.0 nm
P-85	1.0%	18.0 nm
L-64	1.0%	20.4 nm
1:1.5 P-85:L-64	0.01%	17.0 nm
1:2.5 F-68:L-64	1.0%	33.5 nm

EXAMPLE 2 - Fatty Acyl Conjugates

A solution of 50µl of 2 mg/ml of anti-α_2 GP antibody specific for the α_2-glycoprotein of glial cells (Chekhonin *et al.*, *FEBS Lett.*, 287:149-152 (1991)) in 0.1M borate buffer (pH 8.5) was mixed into 2 ml of 0.1M AOT® sodium bis(2-ethylhexyl)sulfosuccinate, available from Serva Chemicals, Germanyl in octane. A reaction is initiated by adding a two-fold molar excess (with respect to the polypeptide) of stearic acid chloride in 0.2 ml of 0.1M AOT® in octane to the mixture. The stearic acid chloride was obtained from stearic acid (available from Reakhim, Russia) as described in Kabanov *et al.*, *Molek Biologiya (Russian)*, 22:473-484 (Engl. edn., 382-391), 1988. The reaction was conducted overnight at 25°C. The product is precipitated three times with cold acetone, dissolved in RPMI 1640 medium and sterilely filtered through a 0.22µm filter (the polygonal antibody used in this experiment also reacted with glial fibrillary acidic protein.).

EXAMPLE 3 - Iodinated Targeting Moieties

Anti-α_2 GP antibody was labeled with ^{125}I using Bolton-Hunter reagent in the system of reversed micelles of AOT® in octane as described in Slepnev V.I. *et al.*, *Bioconjugate Chem.*, 3, 273-274 (1992). Specific radioactivity of the ^{125}I-labeled protein ranges from 19 to 21 Ci/mol.

Wistar rats (80g body weight, 8 animals/group) were injected i.p. (0.1 ml/10g body weight) with a composition made up of the ^{125}I-labeled anti-α_2-GP antibody

(1 mCi/ml) dissolved in a mixture of 1.5% (w/v) copolymer Pluronic P85 and 2.5% (w/v) copolymer Pluronic L64 dissolved in RPMI 1640 medium. ^{125}I-labeled polypeptide dissolved in RPMI 1640 medium was administered at the same concentration. After three days the animals were killed, and tissue samples taken for radioactivity assay to analyze tissue distribution as described by Chekhonin *et al., FEBS Lett.*, 287, 149-152 (1991). The distribution of radioactivity was quantitated by liquid scintillation counting. The experiments were repeated at least twice and the results were reproducible with less than 10% variation. The results, expressed as the ratio of brain radioactivity to the radioactivity in a given tissue (± S.D.), were as follows:

Organ	Relative Content of Label	
	Micelle	Control
Brain/heart	1.22 ±0.91	0.11 ±0.02
Brain/kidney	7.42 ±0.56	0.05 ±0.01
Brain/liver	9.02 ±0.75	0.01 ±0.00
Brain/lung	12.1 ±0.92	0.04 ±0.01
Brain/spleen	6.48 ±0.39	0.01 ±0.00
Brain/blood	8.85 ±0.67	0.01 ±0.00

EXAMPLE 4 - Quantitation of Behavioral Changes

Quantitative evaluation of changes in behavior reactions {See *Theory in Psychopharmacology*, S.J. Cooper, Ed., Vol. 1, (Academic Press, London, New York, 1981) are performed. Groups (10 animals/dose point) of DBA/2 male mice (from Kriukovo Veterinary Department of Russian Academy of Sciences, Russia, 20-25g body weight) with similar characteristics of moving activity are injected i.p. with the test preparations at doses corresponding to 0.10 LD_{95}, Concentrations are adjusted so that a maximum volume of 0.1 ml is injected in each mouse. Mouse mobility (the number of mouse migrations in a cell) and grooming

characteristics are registered for each group at 30 minute intervals over 15 hours using a Rhema Labortechnik device. The experiments are repeated three times.

EXAMPLE 5 - Measuring Toxicity

The lethal effect accompanied by development of specific neurologic symptoms described in *Theory in Psychopharmacology,* S.J. Cooper, Ed., Vol. 1, (Academic Press, London, New York, 1981) is measured. Groups (10 animals/dose point) of DBA/2 mice (18-19g body weight) are injected i.p. with the test preparations. Concentrations are adjusted so that a maximum volume of 0.5 mL is administered to each mouse. For quantitative evaluation of specific lethal action, the lethal dose (L.D.) is calculated using the probit method on the basis of 10 concentration points. The experiments are repeated at least twice and results should reproducible with less than 10% variation.

EXAMPLE 6A - Micelle Formation

A 1:1.5 mixture of Pluronic P85 and Pluronic L64 having individual ratios (n) of (oxypropylene) to (oxyethylene) blocks of 1.00 and 1.50, respectively, and a combined value (N) of 1.30, was diluted with RPMI 1640 medium to a final concentration of 4.0% at 40°C. The mixture was incubated for 30 minutes at 37°C and then sterilized by filtration through a 0.22 μm filter. An equal volume of a solution of 200μg daunorubicin in RPMI 1640 medium was added and this mixture was incubated for 30 minutes at 37°C.

EXAMPLE 6B - Preparation of Brain Targeted Micelles

Equal volumes of the solution of Pluronic micelles of Example 6A and the solution of stearylated antibody of Example 2 were mixed at 37°C. Equal volumes of the resulting solution and a sterile 6 mg/ml solution of haloperidol dissolved in RPMI 1640 were mixed at 37°C.

EXAMPLE 7 - Behavioral Measure of Brain Biodistribution

The preparations described in Example 6, except that the anti-GFAP antibody was not radioactive and was used at a concentration of 0.4 mg/ml, were used in these experiments.

Solutions were administered i.p. Animal mortality was monitored daily for 14 days. The LD_{50} and maximum tolerated dosage ("M.T.D.", *i.e.*, the maximal dose at which no animals among 6 equivalently treated animals died) were calculated by probit analysis. See, Chan and Hayes in *Principles and Methods of Toxicology,* Hayes, A.W., ed., Raven Press, New York, 1989, pp. 169-189. When administered in the Pluronic vehicle, the LD_{95} value of haloperidol was determined to be 0.15 mg/kg, without the Pluronic vehicle, the LD_{95} value of haloperidol was 75 mg/kg.

An amount equaling 10% of the LD_{95} for a given composition was injected i.p. into DBA/2 mice in 0.5 ml of the pluronic vehicle (Example 6). The behavioral results of these injections (± S.D.), measured as described in Kabanov *et al., J. Controlled Release,* 22:141 (1992), were as follows:

Behavior	Micellar form of haloperidol	Free haloperidol
Horizontal mobility	14.4 ± 64%	204.6 ± 24%
Grooming	26.5 ± 76%	1834.8 ± 12.5%

As can be seen from the above table, the micellar form of haloperidol is markedly more active than an amount of free haloperidol normalized at 10% of the LD_{95} amount.

EXAMPLE 8 - Specific and Non-Specific Targeting Molecules

A specific targeting composition was prepared as described in Example 6. The final concentration of the anti-GFAP antibody was 0.02 mg/ml, and its specific radioactivity was 20 Ci/mol.

A non-specific was prepared using the same procedure but substituting a Fab preparation of non-specific murine IgG for the brain-specific antibody. The final concentration of the antibody was 0.02 mg/ml, and its specific radioactivity was 20 Ci/mol.

These preparations (0.5 ml) were injected i.p. into DBA/2 mice. The resulting biodistributions (± S.D.) were:

Organ	Relative Content of label (% Dose/g of tissue)	
	Micelle	Control
Brain	53+4.15*	1.4+0.12
Heart	3.2+0.22	3.1+0.21
Kidney	4.4+0.31	5.1+0.47
Liver	4.3+0.26	36.2+1.92
Lung	2.2+0.11	4.8+0.42
Spleen	4.1+0.33	5.1+0.41
Blood	3.8+0.31	8.7+0.67

EXAMPLE 9 - Targeting Using Neuronal-Specific Anti-Enolase Antibody

A targeting composition was made using the procedure of Example 6 wherein the antibody was a monoclonal antibody against the y-subunit of neuronal-specific enolase ("anti-NSE MAb", available from Russian Research Center, Moscow, Russia). The final concentration of the antibody was 0.35 mg/ml, and its specific radioactivity was 18 Ci/mol. For control experiments, the nonspecific murine antibody preparation described in Example 8 was used.

These preparations (0.5 ml) were injected i.p. into DBA/2 mice. The resulting biodistributions (± S.D.) were:

Organ	Relative Content of label (% Dose/g of tissue)	
	Micelle	Control
Brain	58+5.12*	0.9+0.06
Heart	3.2±0.23	2.8±0.21
Kidney	4.3±0.36	5.6±0.52
Liver	3.8±0.32	31.2±3.05
Lung	2.10±.18	6.4±0.59
Spleen	3.9±0.33	4.9±0.37
Blood	4.1±0.40	7.4 ±0.71

EXAMPLE 10 - Targeting Using Insulin

An insulin targeting molecule was prepared by linking stearyl moieties to insulin (available from Sigma, St. Louis, MO) using the method of Example 6. The targeting molecule was incorporated into a haloperidol composition using the method described in Example 6. The final concentration of insulin in the composition was 0.4 mg/ml. The LD_{95} for this haloperidol composition was determined to be 3.0 mg/kg, using the method in Example 7.

An amount equaling 10% of the LD_{95} for a given composition was injected i.p. into DBA/2 mice in 0.5 ml (6 mice per each treatment). The behavioral results of these injections (±S.D.), measured as described in Kabanov *et al.*, *J. Controlled Release*, 22:141 (1992), were as follows:

Behavior	Micellar form of haloperidol	Free haloperidol
Horizontal mobility	56.1 ±36%	180.1 ± 26%
Grooming	86.6 ± 29%	1656.4 ± 6.5%

As can be seen from the above table, the micellar form of haloperidol is markedly more active than an amount of free haloperidol normalized at 10% of the LD_{95} amount.

EXAMPLE 11 - Sulpiride Compositions

Sulpiride and the stearylated anti-NSE Fab antibody preparation of Example 9 were incorporated into the block-copolymer micelles using the methods described in Example 6. The final concentration of anti-NSE Fab in the preparation was 2.1 mg/ml. A sterile, control solution of sulpiride in RPMI 1640 medium was prepared. The LD_{95} values for the preparations was determined as described in Example 7. For the block copolymer preparation, the LD_{95} was 12.1 mg/kg body weight; for the control preparation it was 100 mg/kg body weight.

EXAMPLE 12 - Trifluorperazine Compositions

Trifluorperazine and anti-GFAP Fab antibody preparation treated with stearoyl chloride were incorporated into the block-copolymer micelles using the methods described in Example 6. The final concentration of antibody in the preparation was 0.2 mg/ml. A sterile, control solution of trifluorperasin in RPMI 1640 medium was prepared. The LD_{95}, values for the preparations was determined as described in Example 7. For the block copolymer preparation, the LD_{95} was 0.04 mg/kg body weight; for the control preparation it was 10 mg/kg body weight.

The minimum neuroleptic dose (MND) was determined for each preparation. The minimum neuroleptic dose is defined as the minimum dose that caused a neuroleptic effect as monitored behaviorally. See, Kabanov *et al.*, *FEBS Lett.*, 258:343-345 (1989). The MND for the copolymer-containing preparation was 0.02 mg/kg, while that of the control preparation was 2 mg/kg. The ratio of LD_{95} /MND was 50 for the copolymer preparation and 5 for the control preparation.

EXAMPLE 13 - Cytotoxicity Against Resistant Cancer Cells

Pluronic P85 was dissolved in RPMI 1640 medium (ICN Biomedicals Inc., Costa Mesa, CA) to a final concentration of 1%, and then the solution was sterilized by filtration to remove bacterial or fungal contamination. This Pluronic P85 solution was used to make appropriate dilutions of sterile drug solutions for the cell culture experiments described below.

The cytotoxicity studies utilized the SKOV3 line of transformed cells (hereinafter "SK cells") and the SKVLB cell line derived therefrom (hereinafter "SK-resistant cells"). Both of these cell lines were provided by Dr. V. Ling, University of Toronto. The SK-resistant cell line is a multi-drug resistant cell line derived from the SK cell line by long term cultivation in the presence of vinblastine.

Various dilutions of a number of anticancer agents were made in RPMI medium or the Pluronic P85 solution described above. Cells were prepared for use in these experiments by plating an equal volume of a cell suspension (2000-3000 cells) into the wells of 96-well microliter plates (Costar, Cambridge, MA) and cultured for 2 days. All cell culturing was done at 37°C and under a 5% CO_2 atmosphere. After this, 100µl per plate of fresh medium (RPMI 1630 medium supplemented with 10% fetal calf serum) was added. The free anticancer agent or copolymer plus anticancer agent dilutions were applied to the wells in 100µl volumes. The cells were exposed to the free or micellar form of a drug for two hours. After this incubation, the cells were washed three times with fresh medium. Then, the cells were cultured under fresh medium for an additional four days.

The number of viable cells for each culture was determined by standard XTT analysis, which measures the activity of mitochondrial enzymes. See, Scudiero *et al.*, *Cancer Res.*, 48:4827 (1988). 50µl per well of sterile 1 mg/ml XTT (2,3-bis[2Methoxy-4-nitro-5-sulfophenyll-2H-tetrazolium-5carboxanilide inner salt, Sigma, St. Louis, MO) in PRMI-1640 containing 5µl/ml of 1.54 mg/ml phenazine

metasulphate (Sigma) in PBS was added to the cells. The cells were incubated for 16 hours, after which the absorbance of each well at 450nm was determined. The SEM for any value determined (the mean of three determinations) was always with 10% of the value. IC_{50} values (*i.e.*, the concentration at which 50% inhibition was achieved) were determined by extrapolating from graphs plotting the number of viable cells (*i.e.*, the mitochondrial enzyme activity) versus the concentration of drug applied to the cells. The results for SK-resistant cells were as follows:

Form of biological agent	IC_{50}, (ng/mi)
Free doxorubicin	60,000
Pluronic L61	70
Pluronic P85	1000
Pluronic F108	2000
Pluronic F68	60,000

EXAMPLE 14 - Copolymer Titrations

The methodology of Example 13 was used except in two details. The first difference was that doxorubicin-resistant MCF7 cells (MCF ADR cells, which described further in Example 21) were used in place of SK cells. Second, in addition to varying doxorubicin concentrations, the concentration of copolymer was also varied: Free doxorubicin; doxorubicin in the presence of 0.61 x 10-6M Pluronic L61; doxorubicin in the presence of 0.3 x 10^{-5} M Pluronic L61; doxorubicin in the presence of 0.16 x 10^{-4} M Pluronic L61; doxorubicin in the presence of 0.8 x 10^{-4} M Pluronic L61; doxorubicin in the presence of 0.4 x 10^{-3} M Pluronic L61; and doxorubicin in the presence of 0.4 x 10^{-1} M Pluronic L61.

EXAMPLE 15 - Parenteral Composition

A composition suitable for parenteral administration was prepared by dissolving 400 mg of Pluronic P-85 and 600 mg of Pluronic L-64 in 50 mL of

RPMI 1640 at 40°C. The mixture was incubated for 30 minutes at 37°C and then sterilized by filtration through a 0.22 gm filter. The filtered solution was mixed with a solution of 100 mg of sterile lyophilized haloperidol powder dissolved in 50 mL of RPMI and incubated for 30 minutes at 37°C.

The composition can be stored in the dark at room temperature for 7 days without loss of activity or can be lyophilized and stored for at least 1 year in the dark at room temperature.

EXAMPLE 16 - Parenteral Composition

A further composition suitable for parenteral administration prepared by dissolving 100 mg of sodium ascorbate in 100 ml of a 9% aqueous solution of sodium chloride. To one-half of this solution were added at 4°C 400 mg of Pluronic P-85 and 600 mg of Pluronic L-64. The mixture was incubated for 30 minutes at 37°C and then sterilized by filtration through a 0.22 μm filter. Separately 100 mg of sterile lyophilized haloperidol powder and 50 mg of glucose were dissolved in the remaining sodium ascorbate-sodium chloride solution and the two solutions were mixed and incubated for 30 minutes at 37°C.

This composition can be stored for 30 days in the dark at room temperature without loss of activity or can be lyophilized and stored for at least 1 year in the dark at room temperature.

EXAMPLE 17 - Parenteral Composition

A further composition suitable for parental administration prepared by dissolving 100 mg of sodium ascorbate in 100 mg of a 9% aqueous solution of sodium chloride. To one-half of this solution were added at 4°C 400 mg of Pluronic P-85 and 600 mg of Pluronic L-64. The mixture was incubated for 30 minutes at 37°C. Separately, 100 mg of lyophilized haloperidol powder and 50 mg of glucose were dissolved in the remaining sodium ascorbate-sodium chloride solution and the two solutions were mixed and incubated for 30 minutes at 37°C. The combined mixture was sterilized by filtration through a 0.22 Am filter. This

composition can be stored for 30 days in the dark at room temperature without loss of activity or can be lyophilized and stored for at least 1 year in the dark at room temperature.

EXAMPLE 18 - Parenteral Composition

A parenterally administrable composition was prepared by dissolving 400 mg of pluronic P-85 and 600 mg of pluronic L-64 in 50 ml of aqueous solution containing 1 mg/ml sodium ascorbate and 0.9 g/ml sodium chloride. The mixture was incubated for 30 min. at 37°C. To this was added 100 mg of lyophilized haloperidol powder and 50 mg of glucose dissolved in 50 ml of aqueous solution containing 1 mg/ml sodium ascorbate and 0.9 g/ml sodium chloride and this combined mixture was incubated for 30 min. at 37°C. To 100 ml of this preparation were dissolved 40 mg of lyophilized hydrophobized anti-GFAP Fab powder and this solution was incubated for 30 minutes at 37°C and then sterilized by filtration through a 0.22 μm filter. The composition can be stored in the dark at room temperature for 30 days without any essential loss of activity or can be lyophilized and stored for at least one year in the dark at room temperature.

EXAMPLE 19

A further composition suitable for parenteral administration is prepared by dissolving 100 mg of sodium ascorbate in 100 ml of a 9% aqueous solution of sodium chloride. To this solution are added at 40°C 10 mg of Pluronic L-61. The mixture is incubated for 30 minutes at 37°C and then sterilized by filtration through a 0.22 μm filter. This solution is packaged together with a container of 10 mg doxorubicin.

EXAMPLE 20 - Acute Toxicity

The acute toxicity of Pluronic F108, P85 and L61 were studies in 5-week old BALB/c male mice. Each experimental group of mice included 6 mice.

Various doses of isotonic Pluronic solutions were administered *i.p.* Animal mortality was monitored daily for 14 days. The LD_{50} and maximum tolerated dosage ("MTD", *i.e.*, the dose at which no animals among 6 equivalently treated animals died) were calculated by probit analysis. See, Chan and Hayes, *Principles & Methods of Toxicology*, Hayes, A.W., ed., Raven Press, New York, 1989, pp. 169-189. The results were as follows:

Pluronic	MTD, g/kg	LD_{50}, g/kg
Pluronic L61	0.1	0.8
Pluronic P85	0.2	0.8
Pluronic F108	5.0	9.0

EXAMPLE 21

The antibodies (Ab) to GFAP and α2-glycoprotein were modified with stearic acid residues as described in Example 1. They were also covalently linked to Pluronic P85 as described by Kabanov *et al.*, J. Controlled Release, 22:141 (1992).

The therapeutic efficacy of doxorubicin in treatment of glioma was explored. C6 glioma cells were inoculated intracerebrally in groups (n=25) of male Sprague-Dawley rats (280-300g) obtained from Kriukovo Department of Nursery of Russian Academy of Sciences. 10, 15, 20, and 25 days after inoculation, (a) 10 mg/kg of free doxorubicin, (b) doxorubicin in 1% Pluronic P85, (c) doxorubicin in 10% Pluronic P85 containing 0.1 mg/ml of Ab modified with stearic acid chloride and (d) doxorubicin in 10% Pluronic P85 containing 0.1 Mg/ml of Ab linked to Pluronic P85 were administered i.p. (volume 1 ml/300g body weight). Controls will be given injections i.p. with an equal volume of saline. Clinical observations were performed daily. Animals were weighted weekly in the first 2 months and monthly thereafter. Vital signs will be verified to ensure that the animal was dead and necropsy was initiated within 5 minutes after the animal died. Data on survival was analyzed to grade the drug effect on tumor incidence and latency.

The data were presented as a ratio of median survival times in the treated group (T) and control (C). For necropsy all major organs were saved and fixed in their entirety. The tail (used in the study for animal identification during in-life phase) was saved in formalin with the animal tissues. All brains were removed and trimmed at three different positions. Three sections of the spinal cord were collected at the cervical, thoracic and lumbar level. Trimmed specimen was placed in Tissue Tek cassettes and processed in a tissue processor. Tissue sections were cut at a thickness of 4-6 mm using a microtome and stained with haematoxylin-eosine. Histopathological examinations of brains assessed: (*i*) the total number of tumors in animals; (*ii*) the number of tumor bearing animals; and *(iii)* the histopathological classification and grading of tumors. The results of the experiment are as follows:

Animal group	Median survival, days	Trial/control x 100%
Control	11.2	-
Free doxorubicin	10.5	-
Micellar doxorubicin	25.3	226
Micellar doxorubicin + strearoylated antibodies	41.0	366
Aicellar doxorubicin + conjugated antibodies	24.5	218

The histopathological examinations also revealed that (1) free doxorubicin caused no effect on tumor size and number compared to control; (2) all 3 micellar formulations caused significant decrease in tumor size and number; (3) the most pronounced effect was observed in the case of micellar doxorubicin + strearoylated antibodies, in this case tumors were practically not observed.

EXAMPLE 22 - *In vivo* Activity of Insulin Formulated During Oral Administration

Hypoglycemia induced by high doses of insulin in mice was used as biological response criteria. The drug activity was evaluated by analyzing the glucose level in plasma versus time following drug administration. Isotonic solutions of free insulin (Ins) or insulin formulated with POE-POP block copolymer ("SP1-Ins") were given to Balb/c mice at the same doses either s.c. or p.o.

Female six-week-old Balb/c mice (six animals per time point) were administered s.c. or p.o. with sterile 100µl per 20g of body weight (5ml/kg) of Insulin or SP1-Insulin solutions, and the same volumes of isotonic solution were given to the control group of animals. Both Insulin and SP1-Insulin injections contained 0.02 mg/ml of insulin with activity of 27.3 u/mg.

The animals were sacrificed after various time intervals (0.5-6hr; post-administration), plasma samples were collected, and glucose levels were analyzed by standard glucosoxidase-peroxidase method. The statistical significance was analyzed by the multiple range text of Duncan-Kramer.

Insulin, when injected s.c., induces a reversible decrease in the glucose level in plasma that reaches about 15% of the normal level 3 hours after drug administration, and then returns to the normal level after about 6 hours. The SP1-Insulin formulation given s.c. produced about the same changes as Insulin (data not shown). The comparison of p.o. administered formulations showed that SP1-Insulin, induces a significant decrease in the glucose level (about 28% of the normal level) with the same pattern of pharmacokinetics as s.c. administered drug, while Insulin given in the same way and at the same dose produces only minor changes.

The results of this study have shown that incorporation of insulin into the block copolymer carriers led to a substantial increase in its activity during oral

administration, suggesting that bioavailability of orally administered SP1-Insulin is comparable to that of s.c. injected free insulin.

EXAMPLE 23

A. A block-copolymer of poly(oxyethylene)-poly(oxypropylene) in which *N* = 1.00 (pluronic P785). is diluted with RPMI 1640 medium to a final concentration of 2.0% at 4°C. The mixture is incubated for 30 minutes at 37°C and then sterilely filtered through a 0.22 μM filter. An equal volume of a sterile solution of human recombinant Interferon-α_2 in RPMI 1640 medium is added, and this mixture is incubated for 30 minutes at 37°C (Prep. A).

B. Antiproliferative activity of Prep. A and nonmodified human recombinant Interferon α_2 solution in RPMI 1640 medium (Prep. B) with respect to Jurkat cells was determined by flow cytometry by a decrease in the index of cell growth (ratio of the number of cells incubated with Prep. A or Prep. B for 24 hours to the initial number of cells). The results obtained are as follows:

Concentration of Interferon-α_2, lg(M)	Index of cell growth ± S.D.	
	Prep A	Prep B
-16	1.68 ± 0.12	1.72 ± 0.11
-15	1.24 ± 0.10	1.71 ± 0.15
-14	1.20 ± 0.12	1.61 ± 0.17
-13	1.14 ± 0.08	1.63 ± 0.13
-12	1.21 ± 0.09	1.44 ± 0.12
-11	1.16 ± 0.06	1.40 ± 0.11
-10	1.20 ± 0.10	1.35 ± 0.12
-9	1.11 ± 0.09	1.28 ± 0.08
-8	1.18 ± 0.10	1.25 ± 0.10

EXAMPLE 24

A. Human recombinant Interferon-α_2 was incorporated in block-copolymer of poly(oxyethylene)-poly(oxypropylene) micelles (N=1.0) as described in Example 23 (Prep. A). Nonmodified human recombinant Interferon-α_2 solution in RPMI 1640 medium (Prep. B) was used as a control. Concentrations of Interferon-α_2 in Prep. A and Prep. B were $1x10^{-13}$M and $1x10^{-10}$M respectively (according to the data represented in Example 23, these concentrations of Interferon-α_2 in Prep. A and Prep B. produce same antiproliferative effect on Jurkat cells).

B. The antiproliferative activity of Prep. A and Prep. B was determined by flow cytometry analysis of the cell cycle distribution of Jurkat cells. The results obtained are as follows:

Sample	Gl/G0, %	S, %	G2+M, %
Control (untreated cells)	50.0	32.5	17. 5
Prep. B	45.0	46.0	9.0
Prep. A	48.0	42.0	10. 0

EXAMPLE 25

A. A 1:1.5 mixture of block copolymers of poly(oxyethylene)-poly(oxypropylene) (pluronics P-85 and L-64) having individual ratios (n) of (oxypropylene) to (oxyethylene) blocks of 1.00 and 1.50, respectively, and a combined value *(N)* of 1.30, is diluted with RPMI 1640 medium to a final

concentration of 2.0% at 4°C. The mixture is incubated for 30 minutes at 37°C and then sterilely filtered through a 0.22μm filter (Prep. A).

B. 50μl of 2mg/ml. natural human Interferon-α_2 in 0.1M borate buffer (pH 8-5) were solubilized in 2ml of 0.1 M AOT® in octane. A 100-fold molar excess (with respect to Interferon-α_2) of stearoyl chloride in 0.1 M AOT® in octane is added to the micellar system obtained. The reaction mixture is incubated overnight at 25°C. Stearoylated cytokine is precipitated three times with cold acetone, dissolved in RPMI 1640 medium and sterilely filtered through a 0.22μm filter (Prep. B).

C. Modified human natural Interferon-α_2 (Prep. B) was incorporated in block-copolymer of poly(oxyethylene)-poly(oxypropylene) in which N=1.30 (Prep-A) as described in Example 24 (Prep. C).

D. Antiviral activity of Prep. C and nonmodified native Interferon-α_2 (Prep. D) used as a control was evaluated by suppression of the cytopathic action of vesicular stomatitis virus on 3T3 NIH cells. Prep. C and Prep. D were added to the cells 24 hours before their infection with a 100-fold lethal close of the virus. Antiviral effect was determined 24 hours after virus administration. Antiviral titer for Prep. C and Prep. D was determined to be $3x10^9$ and $2x10^5$ respectively.

EXAMPLE 26

A. Natural pork interferon alpha was modified with stearoyl chloride as described in Example 25 (Prep. A). Nonmodified native Interferon alpha (Prep. B) used as a control.

B. Antiviral activity of Prep. A and Prep. B was evaluated by suppression of the cytopathic action of vesicular stomatitis virus on kidney cells of pork embryo. Prep. A and Prep. B were added to the cells 24 hours before their infection with a 100-fold lethal dose of the virus. Antiviral effect was determined 24 hours after virus administration. Antiviral titer for Prep. A and Prep. B determined to be 2×10^8 and 1×10^4 respectively.

EXAMPLE 27

A. Natural pork interferon alpha was modified with phosphatidylinositol. To this end, 50μl of 2mg/ml interferon alpha in 0.1 M borate buffer (pH 8.5) are solubilized in 2 ml of 0.1M AOT® in octane. A 50-fold molar excess (with respect to Interferon-α_2) of phosphatidylinositol., oxidized in advance by sodium periodate, in 0.1 M AOT® in octane, and 100-fold molar excess of sodiumborhydride are added to the micellar system obtained. The reaction mixture was incubated overnight at 25°C. The modified cytokine was precipitated three times with cold acetone, dissolved in RPMI 1640 medium and sterilely filtered through a 0.22μm filter (Prep. A). Nonmodified native Interferon alpha (Prep. B) was used as a control.

B. Antiviral activity of Prep. A and Prep. B was evaluated by suppression of the cytopathic action of vesicular stomatitis virus on kidney cells of pork embryo. Prep. A and Prep. B were added to the cells 24 hours before their infection with a 100-fold lethal dose of the virus. Antiviral effect was determined 24 hours after virus administration. Antiviral titer for Prep. A and Prep. B was determined to be $5x10^7$ and $1x10^4$ respectively.

EXAMPLE 28

A. Natural human Interferon-α_2 was modified with stearoyl chloride and incorporated in block copolymer of poly(oxyethylene)-poly(oxypropylene) in which N=1.30 (Prep. A) as described in Example 25 (Prep. A). Nonmodified B is used as a control.

B. Antiviral activity of Prep. A and Prep. B was evaluated by suppression of the cytopathic action of Aujeszky's disease virus on kidney cells of pork embryo. Prep. A and Prep. B were added to the cells 24 hours before their infection with a 100-fold lethal dose of the virus. Antiviral effect was determined 24 virus administration. Antiviral titer for Pre-A and determined to be $1x10^{10}$ and $2x10^5$ respectively.

EXAMPLE 29

A. Human recombinant Tumor Necrosis Factor-α (TNFα) was incorporated in a block copolymer of poly(oxyethylene)-poly(oxypropylene) in which *N*=1.00 (pluronic P-85) as described in Example 23 (Prep. A). Nonmodified TNFα (Prep. B) was used as a control.

B. Specific activity of Prep. A and Prep. B with respect to human ovarian carcinoma $SKOV_3$ cells 48 hours. The results were as follows:

TNF-α concentration, nM	Inhibition, % ±SD	
0.005	Prep. A	Prep. B
0.04	3.3 ± 0.5	2.4 ± 0.5
0.2	24.4 ± 2.7	4.8 ± 1.0
1.0	52.3 ± 4.8	4.8 ± 1.0
5.0	76.7 ± 5.9	8.5 ± 1.2
20.0	84.3 ± 7.9	28.6 ± 2.3
100.0	91.5 ± 8.2	40.0 ± 3.6
150.0	100 ± 11.3	65.0 ± 5.7

EXAMPLE 30

A. Human recombinant Interleukin-2 (IL-2) was covalently conjugated with a poly(oxyethylene)-poly(oxypropylene) block copolymer wherein *N*=1.00 (pluronic P-85) containing terminal aldehyde groups. To this end, 10μg of IL-2 were incubated over 4 hours at room temperature with the 50-fold molar excess of the block-copolymer in the presence of 50-fold molar excess of cyanoborhydride in 0.1 M borate buffer (pH 8.5). The conjugate was purified by gel-filtration on

Biogel P-4 and then incorporated in the micelles of block-copolymer of poly(oxyethylene)-poly-(oxypropylene) in which N = 1.00 (pluronic P85). Example 23 (Prep. A). Nonmodified IL-2 was used as a control (Prep. B).

B. The specified activity of IL-2 in Prep. A and Prep. B was determined using the IL-2 dependent CTLL2 cell line as described by Gillis, *et al.*, *J. Immunol.*, 120: 2027 (1978). The IL-2 activity was equal to $36x10^6$ units/μg in Prep. A and $5x10^6$ units/μg in Prep. B.

EXAMPLE 31

A. Natural human Interferon-α_2 was modified with stearoyl chloride and incorporated in block copolymers of poly(oxyethylene)-poly(oxypropylene) in which N = 1.30 (Prep. A) as described in Example 26 (Prep-A). Nonmodified native is used as a control. Interferon-α_2 (Prep-B).

B. Groups of C57Bl/6-7 week-old male mice which included 36 animals/group were infected (intranasally) with a 10-fold lethal dose of influenza virus H/Chili/1/83 (H1N1). Equal doses of Prep. A and Prep. B were introduced subcutaneously 24 hours after infecting the animals. Survivability of animals was observed during 30 days following drug administration. On the 30th day, the survivability of animals in the control group of nontreated animals was equal to 0%; in the group treated with Prep. A - to 75%; and in the group treated with Prep. B - to 12%.

EXAMPLE 32

A. Natural pork Interferon-α_2 was modified with stearoyl chloride and incorporated in poly(oxyethylene)-poly(oxypropylene) block copolymers in which N = 1.30 (Prep. A) as described in Example 25 (Prep. A). Nonmodified native Interferon-α_2 (Prep. B) was used as a control.

B. Groups of 3-month old white piglets (8 animals/group) not vaccinated against Aujeszky's disease were infected intracerebrally with a 1000-fold LD_{50} of

Aujeszky's disease virus (virulent strain "Arsky"). Prep. A and Prep. B were administered three times intramuscularly: 24 hours before, simultaneously with, and 24 hours after infection in doses of 0.01mg, 0.1mg and 1.0mg per animal per injection. Survivability and Aujeszky's disease symptoms were observed during a 60 day period. In the control experiment the same group of untreated infected animals was studied. The results obtained were as follows:

Sample	Dose (mg per animal)	Survivability in a group, %	Sick rate in a group[a], %
Prep. A	3 x 0.01	100	0
Prep. A	3 x 0.1	100	0
Prep. B	3 x 0.1	0	100
Prep. B	3 x 0.1	12.5	100
Control (untreated cells)	-	0	100

[a]Aujeszky's disease manifestations included disorders of the central nervous system, convulsions, paralysis of gullet, larynx and extremities. The percentage of animals that contracted the disease is presented.

EXAMPLE 33

Prep. A and Prep. B were the same as in Example 31. Groups of 4-month old piglets (11 animals/group) not vaccinated against Aujeszky's disease, were infected intracerebrally with a 10000- fold LD_{50} of Aujeszky's disease virus (virulent strain "Arsky"). Prep. A and Prep. B were administered at the serious stage of the disease three times intramuscularly: on days 6, 8, 10 after infection in the following doses: 0.01 mg, 0.1 mg and 1.0 mg per animal per injection. Survivability and Aujesztky's disease symptoms were observed during a 60 day period. The results were as follows:

Sample	Dose (mg per animal)	Survival rate %
Prep. B	3 x 1.0	0
Prep. A	3 x 0.01	73
Control (untreated animals)	-	0

EXAMPLE 34 - Solution Behavior of Poly(oxyethylene)-Poly(oxypropylene) Block Copolymers

Poly(oxyethylene)-poly(oxypropylene) block copolymers were dissolved in the phosphate-bufferred saline, 10μM, pH 7.4 (PBS) or in 2.5% solution of bovine serum albumin (BSA) in PBS at the concentrations shown below, and the mixtures incubated for at least one hour at 22.5°C or 37°C. The effective diameters of the aggregates formed in these systems were then measured by quasielastic light scattering method as described by Kabanov *et al.*, Macromolecules 28:2303-2314 (1995). The results were as follows:

Copolymer	Conc., %	T, °C	Effective diameter, nm		Comments
			-BSA	+BSA	
Pluronic L61	0.05	22.5	ND	10.6	
	0.1	22.5	ND	23.4	
	0.25	22.5	ND	48.8	
	0.5	22.5	ND	138.3	
	0.005	37	ND	138	
Pluronic L61	0.006	37	ND	-	
	0.008	37	336	-	
	0.01	37	455	120	
	0.025	37	960	(*)	
	0.04	37		(*)	
	0.05	37	1265	(*)	

	0.075	37	1120	(*)	
	0.1	37	LPS	LPS	
	0.25	37	LPS	LPS	
	0.5	37	LPS	LPS	
Pluronic L81	0.04	22.5	-	13.8	
	0.1	22.5	ND	20.6	
	0.25	22.5	ND	379	Very cloudy solution with BSA
	0.5	22.5	935	-	Very cloudy solutions
	0.01	37	-	266	
	0.04	37	1004	(*)	
	0.06	37	(*)	(*)	
	0.08	37	(*)	(*)	
Pluronic L121	22.5	0.01	-	541.5	
	22.5	0.05	-	330	
Pluronic F44	22.5	0.5	ND	12.9	
	22.5	1.0	ND	11.7	
	22.5	2.25	ND	14.2	
	22.5	4.5	ND	28.7	
	22.5	7.5	ND	-	
	22.5	10.0	ND	105	
	37	0.5	ND	84.4	
	37	1.0	ND	97.1	
	37	2.25	ND	137	
	37	5.0	ND	68.1	
	37	7.5	ND		
	37	10.0	12.3	69.4	
Pluronic L64	0.5	22.5	ND	10.8	
	1.0	22.5	ND	12	
	5.0	22.5	ND	21.6	Opalescence and smell fraction of aggregates

					(85 nm) with BSA
	0.1	37	ND	36.2	
	0.5	37	240	192.5	Slightly cloudy solution without BSA and very cloudy solution with BSA
L64 (Cont'd)	1.0	37	16.6	11.6	
	5.0	37	13.1	11.3	
Pluronic P85	22.5	0.5	ND	-	
	22.5	1.0	ND	12.9	
	22.5	5.0	ND	18.7	
	37	0.5	13.9	-	
	37	1.0	12.6	79.6	
	37	5.0	12.8	109	
Pluronic F108	37	2.0	-	22.8	-
Pluronic F127	37	1.0	-	23.2	-
	37	2.0	-	21.5	-
Tetronic T1307	22.5	2.0	-	ND	-
	37	0.5	-	16.7	-
	37	1.0	-	17.1	-
	37	2.0	-	16.6	37.4

"ND": Non Detectable
"LPS": Liquid Phase Separation.
(*) Turbidity was too high for light scattering measurements.

These results suggest that (1) hydrophobic poly(ethylene oxide)-poly(propylene oxide) block copolymers with propylene oxide content not less than 50% (w/v) reveal tendency for aggregation in aqueous solutions at

physiological temperature, (2) aggregation and phase separation of these copolymers is significantly enhanced in the presence of serum proteins.

EXAMPLE 35 - Effects of Hydrophilic Pluronic Copolymers on Solution Behavior of Hydrophobic Pluronic Copolymers

The same procedure as in Example 34, but substituting a mixture of two different poly(ethylene oxide)-poly(propylene oxide) block copolymers for the single copolymer. The results were as follows:

First Copolymer (conc. %)	Second conc., %	T, °C	Effective diameter, nm -BSA	+BSA
Pluronic L121	Pluronic F127 (0.5)	22.5	116.4	
	Pluronic F127 (1.0)	22.5	113.9	
	Pluronic F127 (5.0)	22.5	313.2	
	Pluronic F127 (0.5)	37	88.7	—
Pluronic L121 (0.1)	Pluronic F127 (1.0)	37	77.1	
	Pluronic F127 (2.0)	37	177	
	Pluronic F127 (5.0)	37	262	
Pluronic L61 (0.1)	Pluronic F127 (0.5)	37	26.7	23.8
	Pluronic F127 (1.0)	37	23.6	12.9
	Pluronic F127 (2.0)	37	21.6	13.8
Pluronic L61 (0.125)	Pluronic F127 (1.0)	37	24.7	53
	Pluronic F127 (2.0)	37	22.3	-
Pluronic L61 (0.25)	Pluronic F127 (0.5)	37	(*)	-
	Pluronic F127 (1.0)	37	(*)	-
	Pluronic F127 (2.0)	37	22.4	15.0
Pluronic L61	Pluronic F108	37	840	-

(0.25)	(2.0)			
Pluronic L61 (0.1)	Tetronic T1307 (1.0)	37	(*)	-
	Tetronic T1307 (1.5)	37	915.4	-
	Tetronic T1307 (2.0)	37	16.3	624.8
Pluronic L61 (0.15)	Tetronic T1307 (2.0)	37	387.4	-
Pluronic L61 (0.2)		37	520	-
Pluronic L61 (0.25)		37	735.3	-
Pluronic L61 (0.1)	Tetronic T1307 (2.5)	37	-	522.3
	Tetronic T1307 (3.0)	37		225
	Tetronic T1107 (2.0)	37	(*)	

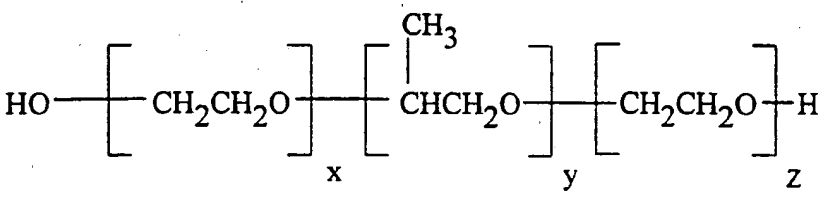
"ND": Non-Detectable.
(*) Turbidity was too high for light scattering measurements.

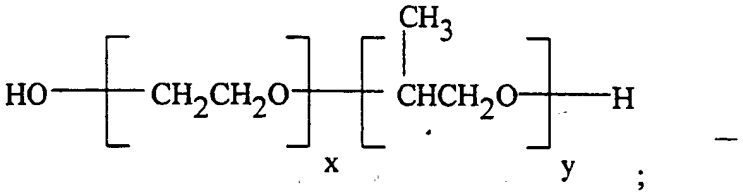
These results suggest that, (1) hydrophilic poly(oxyethylene)-poly(oxypropylene) block copolymers with ethylene oxide content more than 50% (w/v) prevent aggregation of hydrophobic hydrophilic Poly(oxyethylene)-poly(oxypropylene) block copolymers with propylene oxide content not less than 50% (w/v) at physiological temperatures; (2) hydrophilic poly(oxyethylone)-poly(oxypropylene) block copolymers with ethylene oxide content more than 50% (w/v) prevent aggregation of hydrophobic hydrophilic poly(oxyethylene)-poly(oxypropylene) block copolymers with propylene oxide content not less than 50% in the presence of serum proteins.

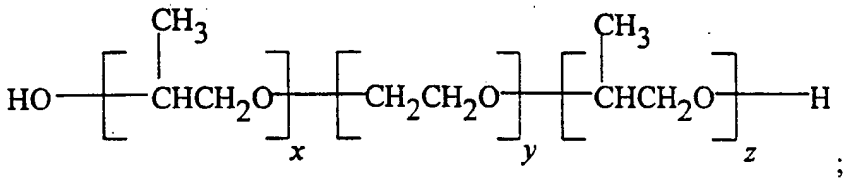
What is claimed:

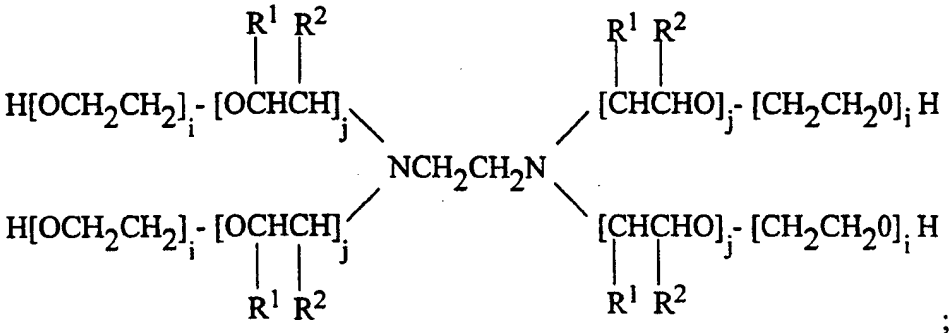
1. A composition comprising a poly(oxyethylene)-poly(oxypropylene) block copolymer and a protein, peptide or derivative thereof covalently modified with a hydrophobe.

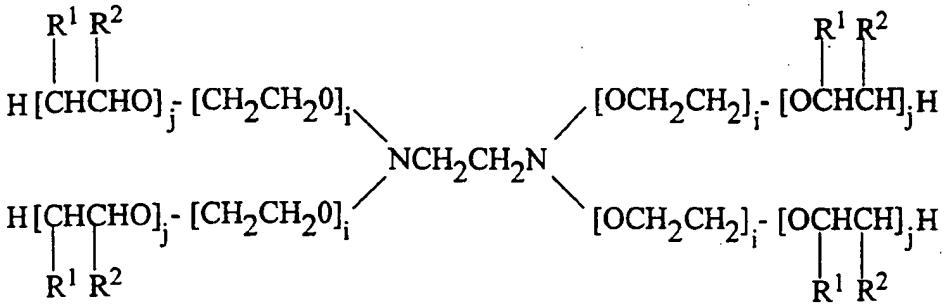
2. The composition according to claim 1, wherein said block copolymer is of the formula:


;


;

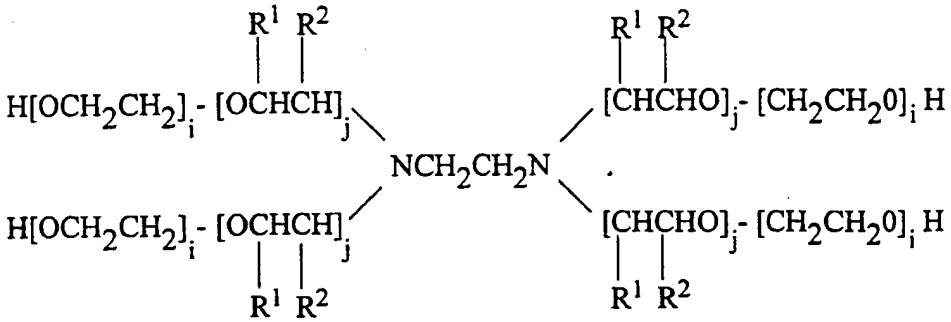

;


; or

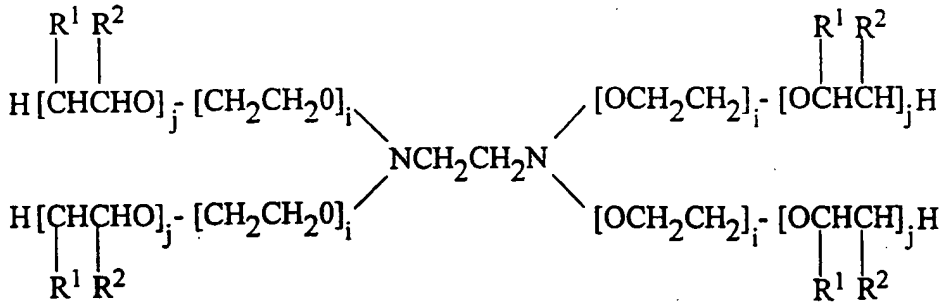


in which x, y, z, i, and j have values from about 2 to about 400, and wherein for each R^1, R^2 pair, one is hydrogen and the other is a methyl group.

3. The composition according to claim 1 wherein said block copolymer is of the formula:

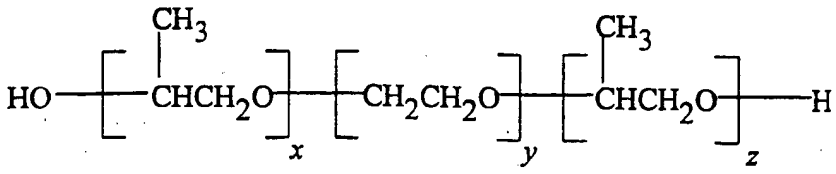


; or



wherein for each R^1, R^2 pair, one is hydrogen and the other is a methyl group.

4. A composition comprising a protein, peptide, or derivative thereof, and a POE-POP block copolymer of the formula:



in which x, y, and z have values from about 2 to about 400.

5. The composition according to claim 1 further comprising at least one block copolymer with ethylene(oxide) content of 50% or less, and at least one block copolymer with ethylene(oxide) content of 50% or more.

6. The composition according to claim 1 wherein said hydrophobe is a lipid.

7. The composition according to claim 1 wherein said hydrophobe is a fatty acid residue.

8. The composition according to claim 1, wherein said protein, peptide, or derivative thereof has a molecular weight of at least about 1,000.

9. The composition according to claim 1, wherein said protein, peptide, or derivative thereof has a molecular weight of at least about 5,000.

10. The composition according to claim 1, wherein said protein, peptide, or derivative thereof has a molecular weight of at least about 10,000.

11. The composition according to claim 1, wherein the protein, peptide or derivative thereof is selected from the group consisting of immunomodulators, cytokines, hormones, enzymes, tissue plasminogen activators, clotting factors, colony stimulating factors, and erythropoetins.

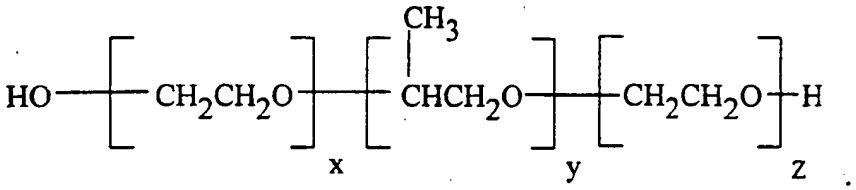
12. The composition according to claim 11 wherein the hormone is a human growth hormone.

13. The composition according to claim 1 wherein the protein, peptide, or derivative thereof is a neuropeptide, or derivative thereof.

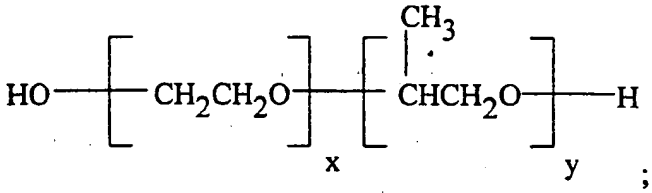
14. The composition according to claim 1 wherein the protein, peptide, or derivative thereof is selected from the group consisting of recombinant soluble receptors and monoclonal antibodies.

15. A method of treatment comprising administering to a patient a poly(oxyethylene)-poly(oxypropylene) block copolymer and a protein, peptide or derivative thereof, covalently modified with a hydrophobe.

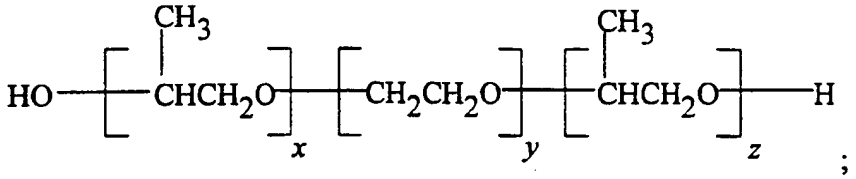
16. The method according to claim 15, wherein said block copolymer is of the formula:



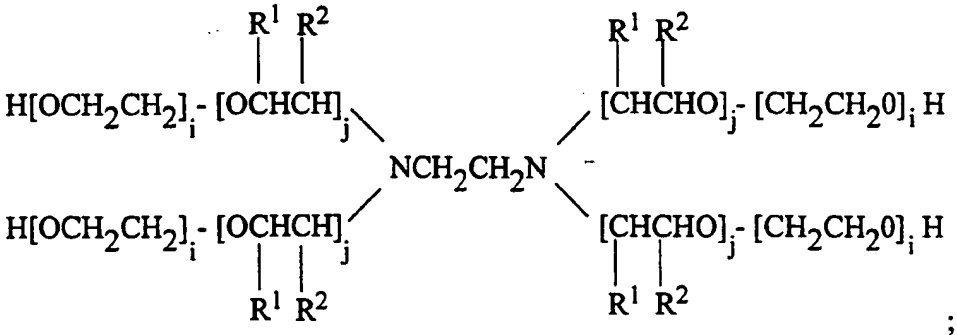
;



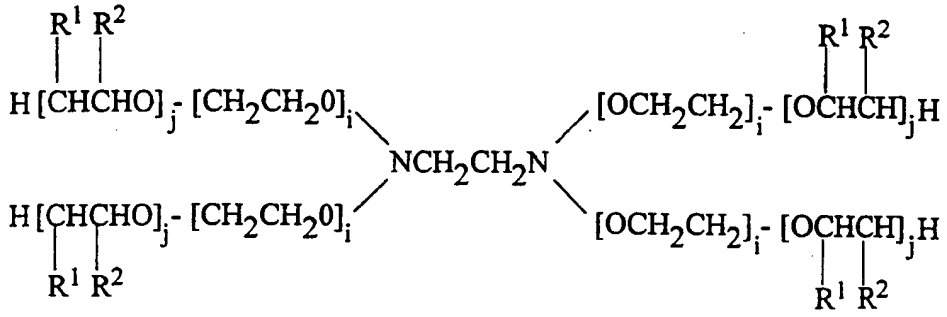
;



;

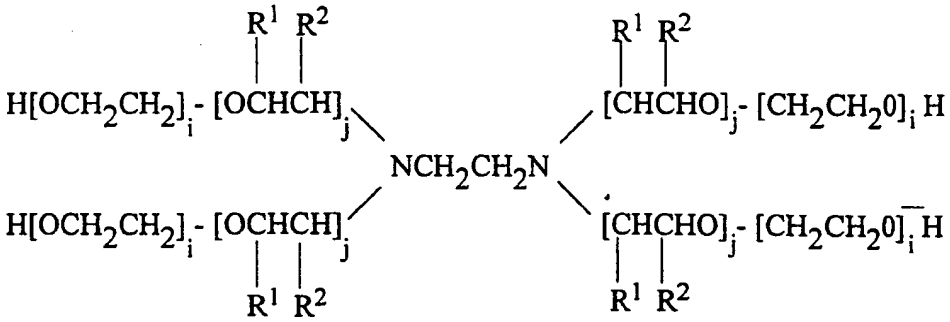


; or

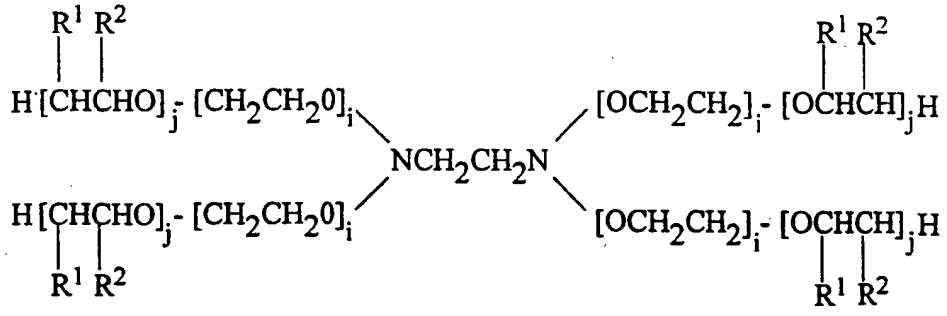


in which *x*, *y*, *z*, *i*, and *j* have values from about 2 to about 400, and wherein for each R^1, R^2 pair, one is hydrogen and the other is a methyl group.

17. The method according to claim 15 wherein said block copolymer is of the formula:



; or



wherein for each R^1, R^2 pair, one is hydrogen and the other is a methyl group, and the ethylene(oxide) content of said block copolymer is less than 50%.

18. The method according to claim 15 further comprising at least one block copolymer with ethylene(oxide) content of 50% or less, and at least one block copolymer with ethylene(oxide) content of 50% or more.

19. The method according to claim 15 wherein said hydrophobe is a lipid.

20. The method according to claim 15 wherein said hydrophobe is a fatty acid residue.

21. The method according to claim 15, wherein said protein, peptide, or derivative thereof has a molecular weight of at least about 1,000.

22. The method according to claim 15, wherein said protein, peptide, or derivative thereof has a molecular weight of at least about 5,000.

23. The method according to claim 15, wherein said protein, peptide, or derivative thereof has a molecular weight of at least about 10,000.

24. The method according to claim 15, wherein the protein, peptide or derivative thereof is selected from the group consisting of immunomodulators, cytokines, hormones, enzymes, tissue plasminogen activators, clotting factors, colony stimulating factors, and erythropoetins.

25. The method according to claim 24 wherein the hormone is a human growth hormone.

26. The method according to claim 15 wherein the protein, peptide, or derivative thereof is a neuropeptide, or derivative thereof.

27. The method according to claim 15 wherein the protein, peptide, or derivative thereof is selected from the group consisting of recombinant soluble receptors and monoclonal antibodies.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04218

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/28, 38/54, 39/395, 45/05
US CL :424/85.1, 94.3, 182.1; 514/3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.1, 94.3, 182.1; 514/3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Derwent/WEST, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,436,170 A (CORNELL et al.) 25 July 1995, see entire document, especially col. 1, lines 3-13 and lines 62-68, and Example 5.	1-11, 14
X - Y	US 5,696,090 A (McGREGOR et al.) 09 December 1997, see entire document.	4 --- 1-3, 5-27
X - Y	US 5,417,982 A (MODI) 23 May 1995, see entire document.	4 --- 1-3, 5-27
X - Y	US 5,554,372 A (HUNTER) 10 September 1996, see entire document.	4 --- 1-3, 5-27

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
18 MAY 1999	02 JUN 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer D. Lawrence for DONNA C. WORTMAN, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04218

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 211 601 A2 (INTERNATIONAL MINERALS AND CHEMICAL CORPORATION) 25 February 1987, see entire document.	1-26
Y	CHEKHONIN, V.P. et al. Fatty acid acylated Fab-fragments of antibodies to neurospecific proteins as carriers for neuroleptic targeted delivery in brain. FEBS Letters. August 1991, Vol. 287, Nos. 1,2, pages 149-152, see entire document.	1-23, 27